0031-3998/88/2405-0600$02.00/0
PEDIATRIC RESEARCH
Copyright © 1988 International Pediatric Research Foundation, Inc.
Vol. 24, No. 5, 1988
Printed in U.S.A.

Precocial Neural Function in the Growth-Retarded Fetal Lamb

C. J. COOK, P. D. GLUCKMAN, C. WILLIAMS, AND L. BENNET

Developmental Physiology Laboratory, Department of Pediatrics, University of Auckland, Auckland, New Zealand

ABSTRACT. Clinical studies suggest that growth-retarded prematurely delivered infants are neurologically precocious. We investigated this paradoxical observation in the fetal lamb. Somatosensory and brainstem auditory-evoked potentials were studied in chronically instrumented fetal lambs in late gestation with varying degrees of growth retardation induced by preconception uterine carunclectomy. The components of the brainstem auditory-evoked response appeared earlier ($p < 0.05$) in fetuses at least 2 SD less than the mean weight for gestational age ($n = 5$) compared to normal controls ($n = 8$) or carunclectomized fetuses of normal size ($n = 7$). Several waveforms of both the somatosensory (N20, P/N 30, and P200) and the brainstem auditory-evoked response (I, III, IV, and V) demonstrated shorter ($p < 0.05$) latencies in growth-retarded fetuses relative to normal-sized fetuses. The ability to follow increasing stimulus rates for both stimuli also demonstrated precocial maturation ($p < 0.05$) in growth-retarded as compared to normal-sized fetuses. Growth retardation is thus associated with precocial neurologic maturation *in utero*. (*Pediatr Res* 24: 600–604, 1988)

Abbreviations

BAEP, brainstem auditory-evoked response
ECOG, electrocorticogram
IUGR, intrauterine growth retardation
SEP, somatosensory evoked response

Clinical studies in the neonate suggest that growth-retarded prematurely born infants are neurologically mature compared to normal sized prematures (1–4). Such infants with evidence of IUGR exhibit shortened brainstem conduction times for auditory stimuli as measured by the BAEP and greater tolerance of the BAEP to increasing stimulus rates (2, 4). The latter observation suggests greater maturation of synaptic function.

These observations suggest that fetal neural maturation is directly affected by the consequences of placental insufficiency. Alternatively they might reflect either the effects of maternal disease such as hypertension or its therapy (2). A further possibility is that fetal development is not affected and that the apparent neural precocity reflects parturition-related events differing in growth retardation and leading to altered neural function in the neonate.

Received April 14, 1988; accepted July 12, 1988.
Correspondence Professor P. D. Gluckman, Department of Pediatrics, University of Auckland, Private Bag, Auckland, New Zealand.
Supported by grants from the Medical Research Council of New Zealand, The Wellcome Trust, and The Neurological Foundation of New Zealand.

Techniques for recording both the SEP and BAEP-evoked potentials in the instrumented fetal lamb *in utero* have been developed (5, 6) and demonstrate developmental changes in a number of characteristic properties of these evoked potentials in the normal-sized fetal lamb over the last 40 days of gestation. We used these indices to examine whether neural maturation is altered in the growth-retarded fetus *in utero*.

MATERIALS AND METHODS

Thirteen pregnant Romney/Suffolk ewes underwent fetal surgery under sterile conditions. The ewes had, at least 6 wk before conception, undergone surgery to remove as many of the endometrial caruncles as possible. Sheep fetuses conceived in ewes after endometrial caruncle removal include a proportion that are acidemic and growth retarded (7, 8). The fetuses, aged between 110 and 113 days at time of surgery, were instrumented for stimulation and recording of both the BAEP and the SEP as previously described (5, 6). Briefly the fetuses had a systemic arterial and venous catheter inserted (carotid or axillary); extradural electrodes were placed to record over the sensory and auditory cortices and to record the biparietal ECOG. A waterproofed earphone was placed over the external auditory monitors after dividing the pinnae that was then sewn over the earphone to hold it in place. Stimulatory electrodes for the SEP were sewn into the upper lip 1–2 cm lateral to the midline and 0.5 cm apart; they were constructed from coaxially insulated stainless steel wire (Cooner Wire Co., Chatswood, CA).

After surgery the ewes were housed in metabolic cages under constant environmental conditions (20° C 50% humidity 12-h light) and fed hay and water *ad libitum*. Supplementation of alfalfa and nuts was also provided. The ewes received antibiotics (not aminoglycosides) prophylactically for 5 days postsurgically and thereafter whenever fetal catheters were opened.

Between 48 and 72 h after surgery stimulation and recording of evoked potentials began. Evoked potentials were obtained from unanaesthetized fetuses *in utero* between 112 and 135 days of gestation (normal term 145–150 days). BAEP were obtained, as previously described (5, 6), using a custom-built stimulator generating a 0.1 ms click at stimulation rates of 1, 15, and 40 Hz at 30 dB above threshold and with amplifier filter settings of 1 Hz–6 kHz. SEP were obtained by stimulation using a custom-built stimulator at 0.1, 0.5, and 2 Hz at 6–8 mAmp above threshold with similar filter settings. Recordings were amplified through custom-built headstages and amplifiers and averaged using a Medelec Sensor, neurophysiologic signal averaging system (Vickers Medical, UK, model ER94a). For the BAEP, 50 ms sweeps were recorded for an average of 256–1028 stimuli. The individual averaged recordings were stored on disc via interfacing to an Apple IIe microcomputer. For the SEP, 5, 50, 100, or 500 ms sweeps were recorded for an average of 1028 stimuli.

In the younger fetus the ECOG has similar characteristics to

the low voltage ECOG state, one of the two ECOG states seen after 125 days (9), and recordings were therefore made only in either the undifferentiated or the low voltage ECOG state. Only the observations made between 125 and 130 days were compared because of the marked gestational effect on the SEP and BAEP (5, 6).

At termination of the experiment fetal weights were obtained and compared to age- and sex-matched fetuses of noncarunclectomized ewes. Premature delivery precluded accurate measurement of placental weights in some fetuses.

RESULTS

Outcome of carunclectomy. Of the 13 fetuses operated upon, one was rejected from the study because of poor postsurgical recovery. The remaining 12 carunclectomized fetuses were considered in three groups; those weighing within 1 SD of the mean of noncarunclectomized fetuses (obtained from 80 fetuses from the same flock also subject to fetal surgery); those 1–2 SD below; and those 2–3 SD below mean weight (Table 1). Evoked potential data from a group of eight noncarunclectomized normal weight for age fetuses of comparable age range (5, 6) were used for comparison. The growth-retarded fetuses were mildly hypoxemic, hypercapnic, and acidemic (Table 1) compared to healthy nongrowth-retarded fetuses of the same age group. This trend reached statistical significance in the most growth-retarded group.

Emergence of waveforms. In normal fetuses the various components of the SEP and the BAEP appear at different but well-defined gestational ages (5, 6). The SEP components appeared at similar ages for all groups of animals during the age range of observation: some of the SEP components emerge earlier than the age range studied here. BAEP components, however, exhibited a precocial appearance ($p < 0.05$) in the most retarded carunclectomized fetuses, whereas the age of appearance in the less growth-retarded fetuses did not significantly differ from noncarunclectomized animals (Table 2).

Amplitude and latencies of waveforms. Certain of the peak and interpeak latencies were shortened in growth-retarded fetuses. For the BAEP the absolute latency of wave I, III, IV, and V and the absolute and interpeak (wave I to V) latency of wave V were reduced ($p < 0.05$) in the 2–3 SD growth-retarded fetuses compared to both carunclectomized and noncarunclectomized normal sized fetuses (Fig. 1; Table 3). Several of the mid-latency SEP components (N20, P/N 30 and P200) also demonstrated shorter ($p < 0.05$) absolute and interpeak latencies in the 2–3 SD growth-retarded fetus with a trend to shortening also observed in the 1–2 SD below mean group (Fig. 2).

The amplitude of the recorded waveforms for both pathways was not significantly different in severely growth-retarded compared to normal-sized fetuses. This suggests a similar population of neurones were activated by the stimuli in both the normal and growth-retarded fetuses (10).

Tolerance of increased rate of stimulation. Growth-retarded fetuses also tolerated greater rates of stimulus presentation without alterations in latencies of the evoked potentials. Both the carunclectomized 1–2 SD and 2–3 SD below normal weight groups tolerated stimulus rates for both the SEP and BAEP that produced significant ($p < 0.05$) changes in latencies in both the carunclectomized 0–1 SD group and noncarunclectomized normal weight fetuses (Fig. 3).

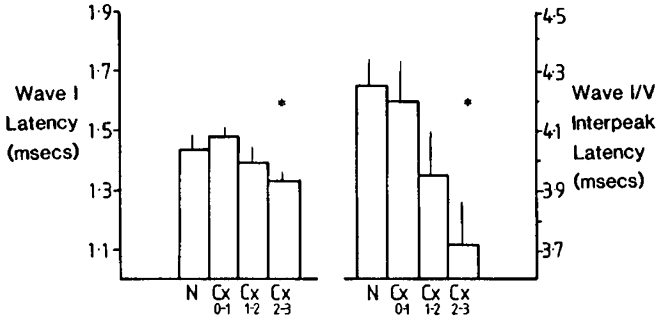

Fig. 1. Representative changes in two components of the BAEP are shown: the onset latency of wave I and the interpeak latency of wave V. Carunclectomized growth-retarded fetuses 2–3 SD smaller than normal mean weight for age (Cx2-3, $n = 5$) showed differences (*$p < 0.05$, analysis of variance) in both components when compared with noncarunclectomized normal weight for age (N, $n = 8$), carunclectomized normal weight for age (Cx0-1, $n = 4$), and carunclectomized growth-retarded fetuses 1–2 SD smaller than normal (Cx1-2, $n = 3$).

Table 1. *Arterial blood gases and pH (mean ± SD)*

	Noncarunclectomized		Carunclectomized	
	$n = 8$	+1 to −1 SD* $n = 4$	−1 to −2 SD $n = 3$	−2 to −3 SD $n = 5$
pH	7.36 ± 0.03	7.35 ± 0.01	7.35 ± 0.04	7.32 ± 0.03
pO_2	19.7 ± 1.1 torr	18.4 ± 2.6 torr	17.8 ± 1.9 torr	14.1 ± 3.7 torr†
pCO_2	45.2 ± 1.5 torr	46.1 ± 1.9 torr	47.0 ± 2.3 torr	51.3 ± 2.1 torr†

* Weight expressed as SD from mean for normal sized fetuses of same gestational age.
† $p < 0.05$ compared to noncarunclectomized.

Table 2. *Age of emergence of BAEP waveforms (gestational age in days)**

Wave	I	II	III	IV	V
Noncarunclectomized ($n = 8$)	17.5 ± 0.5	117.8 ± 0.5	117.8 ± 0.5	119.5 ± 0.9	120 ± 0.8
Carunclectomized +1 to −1 SD ($n = 4$)	117.3 ± 0.5	117.5 ± 0.6	117.8 ± 0.5	120 ± 0	120 ± 0
Carunclectomized −1 to −2 SD ($n = 3$)	117.3 ± 0.6	117.3 ± 0.6	117.7 ± 0.6	119.3 ± 0.6	119.7 ± 0.6
Carunclectomized −2 to −3 SD ($n = 5$)	115.8 ± 0.8†	116.4 ± 0.5†	116.8 ± 0.4‡	118 ± 0†	118.2 ± 0.4†

* Age at which waveform of brainstem auditory evoked response was first observed after stimulation at 0.5 Hz.
† $p < 0.05$ compared to all other groups.
‡ $p < 0.05$ compared to noncarunclectomized and carunclectomized (+1 to −1 SD) groups.

Table 3. *Comparison of BAEP absolute latencies (mean ± SD) between carunclectomized and noncarunclectomized animals (mean ± SD)*

		Noncarunclectomized		Carunclectomized	
	Group		+1 to −1 SD	−1 to 2 SD	−2 to −3 SD
Component latency	I	1.44 ± 0.04	1.45 ± 0.02	1.40 ± 0.08	1.37 ± 0.01*
	II	2.46 ± 0.03	2.43 ± 0.06	2.43 ± 0.06	2.40 ± 0.07
	III	3.53 ± 0.08	3.51 ± 0.01	3.51 ± 0.10	3.40 ± 0.03*
	IV	4.45 ± 0.05	4.44 ± 0.11	4.42 ± 0.05	4.39 ± 0.01*
	V	5.68 ± 0.06	5.59 ± 0.20	5.64 ± 0.09	5.50 ± 0.03*

* $p < 0.05$ compared to noncarunclectomized.

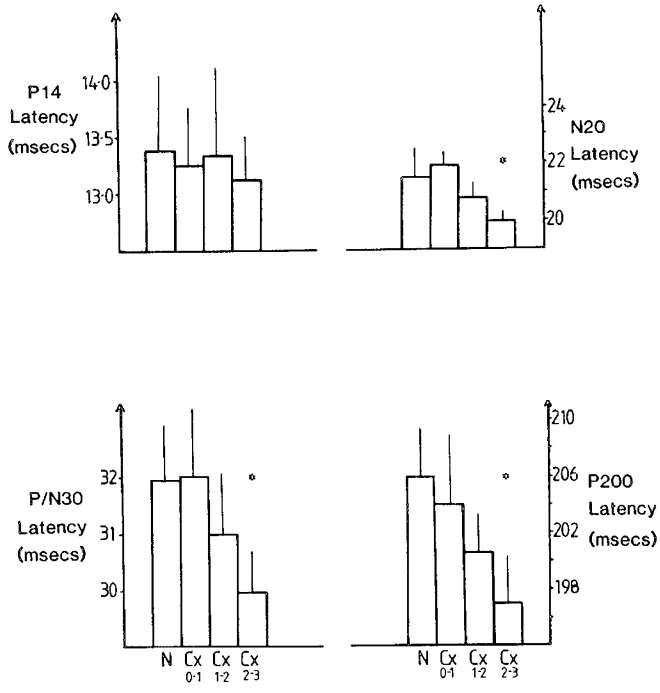

Fig. 2. The onset latency of the SEP components P14, N20, P/N 30, and P200 are shown and the latter three are shorter ($p < 0.05$, analysis of variance) in the carunclectomized most growth-retarded fetuses. Latencies are in ms, labeling is as in Figure 1.

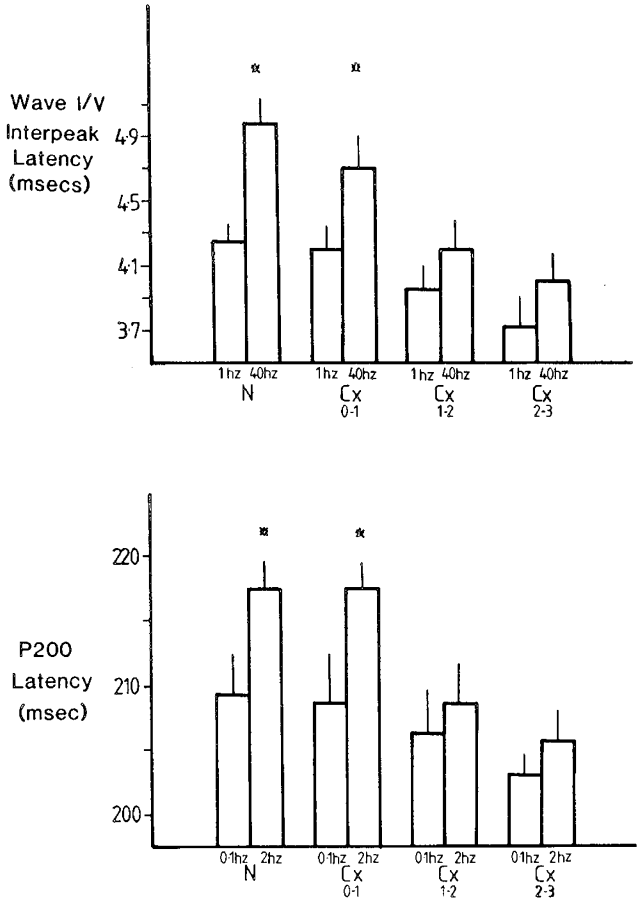

Fig. 3. *Upper panel*, The effect of 1 *versus* 40 Hz on the interpeak latency of waveform V of the BAEP is shown. Groups as in Figure 1. *$p < 0.05$ compared to lower stimulus rate. *Lower panel*, SEP stimulation rates were varied over a range of 0.1 to 2 Hz. Both the 1–2 SD (Cx1-2) and the 2–3 SD (Cx2-3) smaller than normal mean weight groups, tolerated a greater rate of stimulus presentation without alterations in SEP components than the carunclectomized normal weight for age and the noncarunclectomized fetuses. This figure demonstrates changes in the absolute latency (ms) of a representative component (P200) of the SEP, with an increasing stimulation rate from 0.1 to 2 Hz for the four groups. *$p < 0.05$ compared to lower stimulus rate.

DISCUSSION

Infants of mothers with hypertension and born with growth retardation demonstrate accelerated neurologic development as clinically evaluated postnatally (1–4). This paradoxical apparent maturity is not maintained beyond the neonatal period. The question of the role of neural maturation *in utero* in growth-retarded fetus has not previously been addressed; our results suggest that there is altered maturation of sensory systems in the growth-retarded fetus *in utero*. This would suggest that clinical findings in the neonate are not a consequence of parturition-related influences or of maternal disease or drug administration as has been suggested (2), although additional influences due to such factors cannot be excluded.

As shown in our study at least two distinct sensory pathways are affected in severe fetal growth retardation. Further, three distinct aspects of neural function demonstrate altered neural maturation; age of emergence of the BAEP, latencies of the evoked responses, and following frequencies to both stimuli. Each of these alterations in neural function are consistent with an acceleration in the rate of maturation. For the SEP the early latency components, considered subcortical in origin, were not influenced by growth retardation whereas later latency components, considered cortically generated (6, 11) were affected. The auditory components recorded are generated at cochlear, pontine, and collicular levels. Thus both cortical and brainstem functions can be influenced by growth retardation.

Components of the BAEP were detected several days earlier than normal in the most growth-retarded fetuses. Absolute and interpeak latencies BAEP were reduced in the growth-retarded fetuses. As these latencies normally reduce with maturation (5, 6) these observations are consistent with accelerated maturation. Although growth retardation will mean some shortening in pathway length which might explain, at least in part, the earlier appearance of the BAEP and the change in stimulus rate toler-

ance in both pathways cannot be related to pathway length. The greater tolerance to high stimulus rate without either increased latency or loss of amplitude observed in the growth-retarded fetuses is similar to the maturational increase in tolerance seen in normal fetuses (5, 6).

The lack of an effect of growth retardation on the age of appearance of the SEP components suggests that there is not a symmetrical effect of growth retardation on all aspects of sensory maturation or indeed neural development. However, as for the BAEP, certain components of the SEP showed a reduced latency in severe fetal growth retardation. Again the ability to tolerate an increased stimulus rate was greater in the growth-retarded fetus; an effect that is independent of pathway length and is compatible with an acceleration in synaptic maturation within this pathway.

Shortening of wavepeak latencies could be due to a number of factors including increased nerve fiber diameter, increased synaptic efficiency, or a greater degree of myelination. The effect of a reduction in pathway length might also provide a partial explanation. Progressive myelinization is occurring in the fetal lamb brain during the period under discussion (12). Wave I of the BAEP is believed to be generated at the level of the cochlea (10, 13) and accelerated maturation at this level or within the middle ear may lead to the precocial emergence of BAEP waveforms observed in the growth-retarded fetus. However, the reduction in the interpeak latencies of the BAEP and the reduced latencies of latter latency components in the somatosensory pathway suggest effects on both nerve conduction and on synaptic function.

The increased latency in response to increased stimulus rates in the normal fetus is considered to reflect immaturity of synaptic mechanisms such as uptake synthesis and releasing mechanisms which prepare the synapse to function in response to subsequent presynaptic potentials. The greater tolerance to increased stimulus rates in both pathways suggests a direct effect of growth retardation on neural maturation and synaptic function. Similarly studies in the human neonate (4) suggest that growth-retarded human infants demonstrate modified development in synaptic transmission.

The mechanistic basis of the observed changes in sensory neural function is not known. They may be a direct consequence of altered substrate delivery to the fetal brain. The auditory pathway is metabolically very active in the fetal lamb during the gestational age range in which these growth-retarded related changes were observed (14). Cerebral blood flow is similar in normal and growth-retarded fetuses (15) but as circulating concentrations of glucose and amino acids are altered in growth-retarded fetuses, substrate delivery to the brain is different, *e.g.* circulating glucose is reduced whereas alanine levels are elevated (16). Such growth-retarded fetuses have been previously shown to have altered autonomic function, *e.g.* they demonstrate a less sustained brachycardia in response to hypoxia than normal fetuses (8).

A number of hormonal changes are reported in growth-retarded fetuses that may influence neural maturation. Glucocorticoids affect the maturation of many fetal systems and are implicated in neurologic maturation (17, 18). Growth-retarded fetuses demonstrate elevated plasma glucocorticoid levels (7, 8, 16). In growth-retarded sheep and guinea pig fetuses (19) there is a marked elevation in circulating insulin-like growth factor-2 concentrations, a peptide implicated in neuronal development (20, 21). Precocial neural maturation in growth-retarded fetuses occurs despite reduced thyroid hormone concentrations (7, 8).

There is increasing interest in the possible role that neurotransmitters may play in the regulation of neural maturation as distinct from their differentiated function as neural signal modulators. Particular attention has focused on serotonin. Serotonin concentrations in the nervous system at birth have been correlated, across species, with the degree of neural maturation (22). An increased turnover of serotonin and its precursor tryptophan is reported in growth-retarded rats (23, 24) and these rats demonstrate precocial eye opening behavior (23, 25). Other neurotransmitters including noradrenalin and dopamine have also been implicated (26, 27). It seems possible that the turnover of any of these may be affected directly or indirectly by the metabolic or hormonal consequences of placental immaturity.

A question of relevance to these observations is if there is an associated precocial loss of neural plasticity? There are periods of neural maturation (so-called plastic or critical periods) when exposure to external influences determines neural function within particular systems (28–30). Such periods are almost certainly associated with changes in biochemical, morphologic, and/or physiologic properties of neurones and are presumed to follow a developmental timetable. Precocial maturation of neural function in the growth-retarded fetus may mean advancement in this timetable and a consequent mismatch with the normal range of relevant stimuli. Such precocity could thus prevent the obtainment of a full repertoire of neural behaviors, many of which are dependent on postnatal environmental interaction. Also such a loss of plasticity may make the growth-retarded fetus more likely to have residual sequelae after perinatal asphyxia, an outcome that is frequently observed clinically.

REFERENCES

1. Amiel-Tison C 1980 Possible acceleration of neurological maturation following high risk pregnancy. Am J Obstet Gynecol 138:303–387
2. Henderson-Smart DJ, Pettigrew AG, Campbell DJ 1983 Clinical apnoea and brainstem neural function in preterm infants. N Engl J Med 308:353–357
3. Hadi HA 1984 Fetal cerebral maturation in hypertensive disorders of pregnancy. Obstet Gynecol 63:214–219
4. Edwards DA, Pettigrew AG, Henderson-Smart DJ 1987 Analysis of neural transmission in the brainstem of preterm infants. In: International Symposium on Fetal and Neonatal Development. St Catherines College, Oxford, p 34
5. Cook CJ, Williams C, Gluckman PD 1987 Brainstem auditory evoked potentials in the fetal sheep in utero. J Dev Physiol 1:379–398
6. Cook, CJ, Gluckman PD, Johnston BM, Williams C 1987 The development of the somatosensory evoked potential in the unanaesthetised fetal sheep. J Dev Physiol 9:441–455
7. Robinson JS, Jones CT, Thorburn GD 1979 Studies on experimental growth retardation in the sheep. The effect of removal of endometrial caruncles on fetal size and metabolism. J Dev Physiol 1:379–398
8. Robinson JS, Jones CT, Kingston EJ 1983 Studies on experimental growth retardation in sheep. The effects of maternal hypoxaemia. J Dev Physiol 5:89–100
9. Clewlow F, Dawes G, Johnston B, Walker D 1983 Changes in breathing electrocortical and muscle activity in unanaesthetised fetal lambs with age. J Physiol 341:463–470
10. Chiappa KM 1983 Evoked Potentials in Clinical Medicine. Raven Press, New York
11. Persson H 1973 Development of somatosensory cortical functions—an electrophysiological study in prenatal sheep. Acta Physiol Scand [Suppl.] 395:1–64
12. Patterson D, Sweasey D, Hebert C 1971 Changes occurring in the chemical composition of the central nervous system during foetal and postnatal development of the sheep. J Neurochem 18:2027–2040
13. Church M, Williams H, Holloway J 1984 Brainstem auditory evoked potentials in the rat: effects of gender, stimulus characteristics and ethanol sedation. Electroencephalogr Clin Neurophysiol 59:328–339
14. Abrams R, Ho M, Frisinger J, Patlak C, Pettigrew K, Kennedy C 1984 Local cerebral glucose utilisation in fetal and neonatal sheep. Am J Physiol 246:R608–R618
15. Clapp JF, McLaughlin MK, Gellis J, Hemphill C, Catalano PM 1984 Regional distribution of cerebral blood flow in experimental intrauterine growth retardation. Am J Obstet Gynecol 150:843–846
16. Harding JE, Jones CT, Robinson JS 1985 Studies on experimental growth retardation in sheep. The effects of a small placenta in restricting transport to growth of the fetus.
17. Gould JB, Gluck L, Kulovich MV 1977 The relationship between accelerated pulmonary maturity and accelerated neurological maturity in certain chronically stressed pregnancies. Am J Obstet Gynecol 127:181–186
18. De Lemos RA 1975 Glucocorticoid effect: organ development in monkeys. In: Moore TD (ed) Lung Maturation and Prevention of Hyaline Membrane Disease, p 77
19. Bassett N, Gluckman PD 1987 Insulin-like growth factors in experimental fetal growth retardation in the sheep fetus. Endocrinology 120:234A
20. Sara VR, Hall K, Von Holtz H, Humbel R, Sjogren B, Wetterberg L 1982 Evidence for the presence of specific receptors for insulin-like growth factor 1 and 2 and insulin throughout the adult human brain. Neurosci Lett 34:39–44
21. Haselbacher GK, Schwab ME, Pasi A, Humbel RE 1985 Insulin-like growth factor II (IGF-II) in human brain: Regional distribution of IGF-II and of

higher molecular mass forms. Proc Natl Acad Sci USA 82:2153–2157
22. Baker PC, Quay WB 1969 5-Hydroxytryptamine metabolism in early embryogenesis and the development of brain and retinal tissues. Brain Res 12:273–295
23. Hole K 1972 Reduced 5-hydroxyindole synthesis reduces postnatal brain growth in rats. Eur J Pharmacol 18:361–367
24. Lauder JM, Krebs H 1976 Effects of p-chlorophenylalanine on time of neuronal origin during embryogenesis in the rat. Brain Res 107:638–647
25. Lubchenco L, Hansman C, Dressler M 1963 Intrauterine growth as estimated from live born birth weight data at 24–42 weeks of gestation. Pediatrics 32:793–797
26. Ahmad G, Zamenhof S 1978 Serotonin as a growth factor for chick embryo brain. Life Sci 22:963–970
27. Sievers J, Berry M, Baumgarten H 1981 The role of noradrenergic fibres in the control of post-natal cerebellar development. Brain Res 207:200–208
28. Killackey HP 1985 Intrinsic order in the developing rat trigeminal system. In: Rowe M, Willis WD (eds) Development, Organisation and Processing in Somatosensory Pathways, vol 14. Neurology and Neurobiology. Alan R Liss Inc. NY.
29. Hubel DH, Wiesel TN 1977 Functional architecture of macque monkey visual cortex. Proc R Soc Lond [Biol] 198:1–59
30. Blakemore C, Vital-Durand F 1981 Postnatal development of the monkey's visual system. In: The Fetus and Independent Life. Ciba Foundation Symposium 86. Pitman, London, pp 151–171