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Effect of intrauterine positions on fetal brain development in the rat

STEPHEN ZAMENHOF and EDITH VAN MARTHENS

Brain Research Institute and Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024 (U.S.A.)

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The effect of the position on the horn on fetal brain development was studied in 2276 rats (248 litters), by assessing the intrauterine location of those fetuses which had the highest values ('maximal fetuses') of the following parameters: body weight, brain weight, brain DNA (cell number), brain protein and the ratios brain weight/body weight and brain protein/brain DNA. The fetuses were at term (21 days) or at 16–18 days postcoitum. The total numbers of animals in the right and in the left uterine horn were essentially the same: 4.64 and 4.54, respectively. For each parameter, the locations of maximal fetuses were, on the average, symmetrical with respect to the center of each horn. The distributions of maximal fetuses along each horn followed specific patterns, which were different for each horn but showed similar locations of peaks and depressions for various parameters. However, the differences between the peaks and depressions were not statistically significant. It is concluded that (1) both horns are equipotent, and (2) all the positions on the horn are also essentially equipotent for production of animals maximal with respect to parameters studied. This also suggests that occasional cases of outstanding high fetal brain development occur from causes other than a favorable position on the uterine horn.

The effect of intrauterine position on prenatal growth has been the subject of a few publications[1–3,5–7]. McLaren and Michie reported that in mice the lightest fetus is in the middle of the uterine horn[7], or at the cervical end of the horn[6]. McLaren[5] has also reported that the right uterine horn in mice contains, on the average, more fetuses than the left. This was also reported by Barr et al.[1,2]; however, these investigators claimed that, in the rat, the heaviest fetuses occupied the middle of the uterine horn, whereas the lightest were at either end. Fetal weights reported by Barr et al.[1,2] were highly variant, probably because nominal fetal age is only an estimate and the actual fetal ages at the time of caesarian section (needed to examine positions of individual fetuses) may vary from one pregnant animal to another; thus, it is not safe to compare fetal weights from one mother with those from another.

In all of the previous investigations, fetal brain parameters were not studied.

In the present work, we have used a different approach. Rather than take averages of fetal weights from different mothers, we identified the heaviest fetus of each litter ('maximal fetus') and we determined its location in the right or in the left horn. We have also determined weights, DNA (or cell number) and protein contents of cerebral hemispheres (brain) and similarly, we have identified and located in each litter fetuses which had the highest values of these parameters (maximal fetuses). The fetuses with highest values of the ratios brain weight/body weight and brain protein/brain DNA (index of brain cell size) were also identified and located. We have also compared the total number of fetuses of right horns vs left horns, as well as the proportions of right horns or left horns containing maximal fetuses. The symmetry and pattern of distribution of these maximal fetuses along the horns were also studied. In our work we have used a simplified logical procedure to estimate the location (distribution) of the maximal fetuses, as described below.

The rats (total of 2,276; 248 litters) were as de-

Correspondence: S. Zamenhof, Brain Research Institute and Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024, U.S.A.

scribed in the previous work[8,10]; they were Sprague–Dawley-derived, and were bred in our closed colony for 42 generations. Three-month-old virgin females weighing 200–260 g were mated; the presence of a vaginal plug was considered day 0 of pregnancy. The rats were fed a pelleted diet (Wayne Mousebreeder Block, Allied Mills, Chicago, IL) containing 20.5% protein.

All fetuses were delivered by caesarean section on the 16th–18th or 21st day of pregnancy. All viable fetuses were blotted dry, weighed, decapitated, and their cerebra (without cerebellum and olfactory lobes) were dissected out, weighed and stored at −10 °C for subsequent analysis. DNA was determined by a modification of a diphenylamine colorimetric method[9]; protein was determined by a modification of the Lowry colorimetric method[4].

To determine the location of any fetus along each uterine horn, the following method was used. In each horn, the ovarian end was designated as 0%, and the cervical end as 100% of the length of this horn. The actual figures for each fetal position depend, of course, on the number of fetuses in this horn. The mean distribution was assumed to be uniform (equidistant): it would be practically impossible to check deviations from uniformity in each case, and it is assumed that on the average, any such deviations would even out. Thus, for instance, in a horn containing 3 fetuses, they would be designated as situated at distances 25, 50 and 75% (of the horn length) from the ovarian end; in a horn containing 4 fetuses, the distribution would be 20, 40, 60 and 80%, etc. Fetus with highest value of a parameter under study (e.g. brain weight) ('maximal fetus') was identified in a litter and the position of *such a fetus only* was recorded (Table I). The same procedure was repeated for each litter and each parameter studied. In most cases, there was only one such maximal fetus per litter, i.e. it was a fetus either in the right or in the left horn. However, occasionally two fetuses in the same or in opposite horns had the same maximal value of a parameter, and in such cases both were recorded. Thus, the total proportion of horns with maximal fetuses is somewhat higher than 50% (Table I, bottom part).

For graphic representation of the distribution of maximal fetuses along each horn, we have divided each horn into segments: 10–20%, 20–30%, 30–40%, etc., of total horn length (starting from the ovarian end), and we counted total number of cases of maximal fetuses occurring per segment (Figs. 1–3).

The results are represented in Table I and on Figures 1–3.

The total number of litters examined was 248, the

TABLE I

Mean locations of fetuses with highest values of parameters ('maximal fetuses')

Mean locations are weighted mean distances of maximal fetus from ovarian end of uterine horn, in % of total length. R, right and L, left uterine horn. Proportion of horns with maximal fetuses is total number of horns with maximal fetuses, divided by total number of litters (248). Means are of first 4 parameters, ± S.D.; *P*, probability, n.s., not significant (Student's *t*-test).

	Horn	*Parameters*				*Ratios*	
		Body weight	*Brain weight*	*Brain DNA*	*Brain protein*	*Brain wt./ body wt.*	*Brain protein/ DNA*
16–18 days	R	53.5	52.39	50.44	50.34	47.00	47.15
	L	54.41	47.29	48.75	50.09	48.74	48.42
21 days	R	51.45	51.91	48.66	49.75	47.26	48.58
	L	50.70	49.78	44.10	49.96	51.07	48.43
All ages	R	51.90	52.03	49.03	49.88	47.21	48.24
	L	51.75	48.98	45.48	50.00	50.41	48.43
Total	R + L	51.82	50.50	47.26	49.94	48.78	48.33
Mean	R + L	49.9 ± 1.9					
Proportion of horns	R	0.61	0.56	0.59	0.55	0.59	0.56
with maximal fetuses	L	0.59	0.61	0.66	0.55	0.57	0.52
Mean	R	0.58 ± 0.03					
	L	0.60 ± 0.04					
P		n.s.					

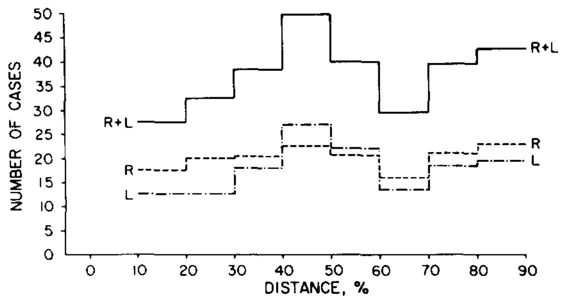

Fig. 1. Distribution of cases of highest fetal body weight ('maximal fetuses') along uterine horns. R, right horn, L, left horn, R + L, total. Term (21 days) and 16–18 days together. Ordinate, numbers of cases; abscissa, distance of maximal fetus from ovarian end of uterine horn, in % of total length of this horn.

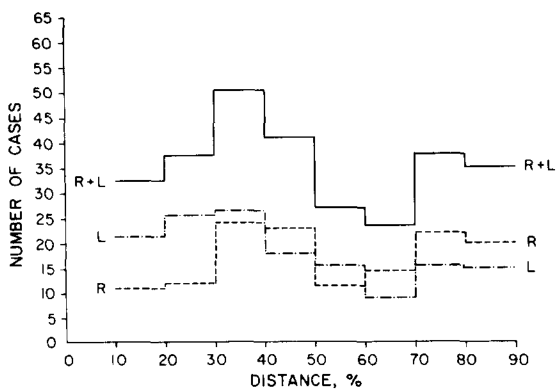

Fig. 2. Distribution of cases of highest fetal brain weight (maximal fetuses) along uterine horn. Other details as in Fig. 1.

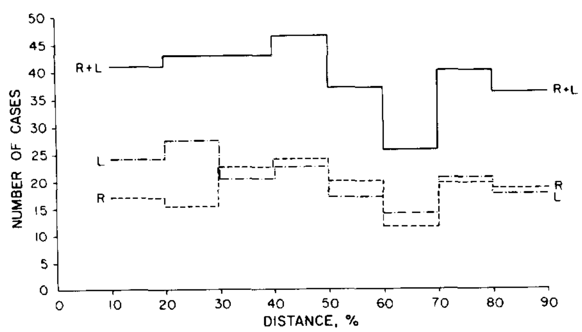

Fig. 3. Distribution of cases of highest fetal brain DNA (cell number) (maximal fetuses) along uterine horn. Other details as in Fig. 1.

total number of fetuses 2276, of which 1151 were in the right horn and 1125 in the left. Thus, the mean litter size was 9.18, and number of fetuses was 4.64 per right horn and 4.54 per left horn. However, due to great variability (range 0–10 fetuses per horn) this small difference is statistically not significant: we conclude that both horns are essentially equipotent.

The same conclusion was reached when we studied the number of 'maximal fetuses' in each horn (Table I, bottom part): it can be seen that for all 4 parameters studied (body weight, brain weight, brain DNA (cell number) and brain protein) and for their ratios brain weight/body weight, and brain protein/brain DNA (index of protein per cell and of cell size) the proportion of horns that had fetuses with highest values of these parameters is practically the same for the right and the left horn. The small difference between horns was statistically not significant. Thus, both horns are equipotent also in their ability to produce 'maximal fetuses'.

As can be further seen from Table I (upper part), the mean distance of the maximal fetus from the ovarian end is very close to 50% of the length of the horn. This means that, on the average, the maximal fetuses are distributed symmetrically with respect to the center of the horn (50%). This is true for all parameters studied, including the ratios brain weight/body weight and brain protein/brain DNA.

The above result (symmetry of distribution) suggests that not only are both horns equipotent, but also the first and second half of each horn are equipotent in their ability to produce maximal fetuses.

It can be further seen from Table I that the results for 16–18-day fetuses and for term fetuses are essentially the same, suggesting that the above postulated equipotency is established early in fetal life.

The distributions of cases of 'maximal fetuses' along uterine horns for 3 parameters are represented on Figs. 1–3. The resulting patterns do not follow normal distribution curves. The patterns for right and left horns are not significantly correlated. For all parameters studied, the total numbers of cases (right plus left horn) exhibited a peak between 30 and 40 or 40 and 50% of the length of the horn, and a depression between 60 and 70%. However, the differences between peaks and depressions are small, and because of high variability from one litter size to another, they are not statistically significant (e.g. comparing brain weights for both horns across all litter sizes, differences between number of cases in 30–40% range vs 60–70% range: Student's t-test: $t = 0.38$; $P > 0.7$). It must be concluded that for production of a 'maximal fetus', all locations on both horns are practically equipotent.

In the previous work[10] we have identified neonatal rats with outstanding high values of brain parameters (more than 2 S.D. above the mean). The causes of such high values were not genetic but were ascribed to outstanding favorable intrauterine conditions, essentially unknown. The present work suggests that the effects of intrauterine positions contribute little to these favorable conditions, and that the causes of outstanding high fetal brain parameters must be sought elsewhere.

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