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Final number of Purkinje and other large cells in the chick cerebellum influenced by incubation temperatures during their proliferation

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Cerebral cell numbers at birth (mammals) or at hatching (birds) tend to remain uniform[11] within the species and the strain; however, occasionally, they may undergo considerable deviations, presumably due to variations in the environmental conditions during the sensitive proliferation period of these cells. The deviations may be spontaneous or induced[11]. Of the latter, the *decrease* in the number of cerebral (review in ref. 11) or cerebellar[1,2,7] cells in mammals, induced by prenatal or postnatal malnutrition, has been frequently reported. On the other hand, the *increase* has been demonstrated only in special cases (review in ref. 11), and *a priori* one might suspect the presence of some regulatory mechanism that tends to limit this cell number.

In order to obtain some information on this subject, in a previous work[9] we incubated chick embryos at an elevated temperature (40.5 °C) on days 7–10, and at a temperature optimal for hatching (37.5 °C) at other times; their optic lobes and cerebral hemispheres at day 10 and at hatching were compared with controls incubated at 37.5 °C only. Cell numbers at day 10 were directly counted by a new method involving formalin fixation and cell disaggregation by gentle sonication. At hatching, body weights, organ weights and organ DNA (cell number) were the same in experimentals and in controls, for both optic lobes and cerebral hemispheres. However, at 10 days (end of neuron proliferation) the weights and the cell numbers in experimentals were significantly higher. Two possible hypotheses have been offered. (1) Elevated neuron population in experimental animals at day 10 is followed by their elevated death rate. (2) There is a net increment in the permanent neuron number but at hatching it is overshadowed by the population of other cells.

In the present work we have obtained evidence in support of the second of the above hypotheses. We have devised the following system. (1) The environmental conditions (temperature) are changed only during a short period (days 5–7 of embryonal life) which coincides with the proliferation of essentially only three types of cerebellar neurons: Purkinje, Golgi II and large neurons of the central cerebellar nuclei. (2) The cell types chosen permit their unequivocal quantitative identification due to their large size. (3) These neurons are counted when their number becomes

essentially final (functionally mature cerebellum which is already present right after hatching in the precocial birds such as chick).

The eggs and their handling were essentially as described in our previous work[9,10]. Fertile eggs (White Leghorn strain K 137, from 10-month-old hens) were supplied by the Kimber Farms, Pomona, Calif.; the experimental and control groups were matched for egg weight. The eggs were incubated at 37.5 ± 0.1 °C (controls) or at 37.5 ± 0.1 °C during the first 4 days and after day 7, and at 40.5 ± 0.1 °C or 35.3 ± 0.1 °C during days 5–7 (experimentals). For direct cell enumeration at hatching, the cerebella were dissected out, weighed and preserved at room temperature in 10% buffered formalin for a period of 10 days to 1 month. They were then disintegrated in 2 ml water in a conical tube, using a glass rod tipped with a rubber policeman. The coarse suspension was then subjected to ultrasonication[6] for 4 min at 55 W, using a microprobe of 3.5 mm diameter tip. The uniform fine suspension was then diluted 1:2 and stained with 0.005 ml of 1% thionine solution; the Purkinje, Golgi II and central cerebellar nuclei cells (together), easily identified by their large size, and separately all the other cells were then counted in a 0.1 mm deep Spencer–Neubauer hemacytometer, using a magnification of 430 in the microscope. All countings were made in a 'blind' fashion, without knowing to which group the sample belonged. The erythrocytes (approx. 1.5%), epithelial cells and blood vessel cells, easily identified under the microscope, were not counted.

The results are represented in Table I. As expected, the deviation from optimal

TABLE I

CEREBELLA OF CHICK EMBRYOS INCUBATED AT DIFFERENT TEMPERATURES

Different batches of eggs were used in experiments A and B. All values ± standard deviation. Large neurons: P, Purkinje: G, Golgi II; ccn, central cerebellar nuclei.

Experiment	*Incubation*	*Body weight (g)*	*Cerebellum*		
			Weight (mg)	*Cell number* × 10^{-5}*	
				P + G + ccn	*Total*
A	Control (37.5 °C)	41.14 ± 3.32	106.35 ± 6.49	1.71 ± 0.178	789.7 ± 97.76
	Experimental (40.5 °C, days 5–7)	39.16 ± 2.19	104.45 ± 10.13	2.08 ± 0.257	786.7 ± 88.13
	Δ**	—4.8	—1.8	+22	—0.4
	Probability***	n.s.	n.s.	< 0.0005	n.s.
B	Control (37.5 °C)	42.4 ± 4.6	105.8 ± 14.2	1.79 ± 0.365	742.8 ± 119.5
	Experimental (35.3 °C, days 5–7)	44.5 ± 3.12	111.3 ± 9.97	1.53 ± 0.035	792.6 ± 41.5
	Δ**	+4.9	+5.2	—14.6	+6.7
	Probability***	n.s.	n.s.	< 0.01	n.s.

* Average of 6 counts per cerebellum. Twelve animals used for each incubation.
** Δ, difference to control, in per cent of control.
*** Student's *t*-test; n.s., not significant.

temperature of incubation during days 5–7 only did not significantly affect the proliferation of those cerebellar cells whose period of proliferation occurs after day 7; such cells constitute over 99% of the total neural cell population of the cerebellum (Table I). Consequently, cerebellar weight also remained unchanged. In contrast, Purkinje, Golgi II and large neurons of the central cerebellar nuclei whose main proliferation period coincides with days 5–7 (refs. 3–5) were influenced by the temperature of incubation during this period: temperatures higher than optimal increased this number, and lower than optimal decreased it; the changes were statistically highly significant. Since in these precocial birds (chick) the cerebellum is mature and functional at hatching, these increments, measured at hatching, may be considered final.

The acceleration or deceleration of the proliferation of embryonal cells in general, by the changes of incubation temperature, is known (review in ref. 8). However, the indication emerging from the present study is that, at least in the case of these neurons, the induced changes in their number are not (or not fully) eliminated, until the maturity of the cerebellum. Thus, the final number of such class of neurons may be increased or decreased depending on the conditions, such as temperature, prevailing during the sensitive period of their proliferation.

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