Brain Growth and Learning Behaviour of the Guinea-Pig Following Prenatal Hyperthermia

Hyperthermia induced at specific stages of pregnancy in guinea-pigs and rats has resulted in a wide range of congenital abnormalities, particularly affecting the central nervous system[1-4]. A deficit of 26–28% in the wet brain weight of newborn guinea-pigs followed maternal exposure to an environmental temperature of 42.5 °C for 1 h daily on days 18–25 of gestation. Exposure between days 39–46 and 53–60 resulted in a less severe brainweight deficit in the newborn. The most sensitive stage of development was about days 20–23. The extent of the deficit depended also on the degree of maternal hyperthermia and number of exposures given[3]. The smaller brains of newborn from heat-stressed mothers contained less DNA than those from control animals indicating a reduction in cell numbers[5]. As hyperthermia was induced probably while neuronal proliferation was taking place, the deficit in cell numbers might have included a deficit of neurones. The most rapid increase in brain size of guinea-pigs occurs between day 45 of gestation and day 10 of post-natal life[6], and although hyperthermia was applied well before this phase of development it was still able to modify the extent of the 'growth spurt'.

Newborn guinea-pigs with severe retardation of brain development were clumsy, slow in movement or incoordinated and unresponsive to external stimuli but less severely affected individuals were not detected by their neonatal behaviour except to appear slightly less active. The experiments to be described were designed to test whether the deficit in brain size at birth could be made up during post-natal life, and whether the learning ability was affected.

Female guinea-pigs were exposed to temperatures of 42–43 °C dry bulb, and 22–27 °C wet bulb in a forced-draught electric egg-incubator, for 1 h daily on days 20–24 (Group 1), 40–44 (Group 2) or 56–60 (Group 3) of gestation as previously described[2]. Each treated group of females was matched with an unheated control group. The mothers were isolated on day 60 of gestation and after littering the newborn were identified by numbered metal tags which were placed in each ear. The young were reared in open pens containing 30 to 40 animals.

At approximately 100 days of age, 10 or 12 offspring from both control and heated mothers of each treatment group were selected for testing on a non-spatial discrimination reversal task. Following a preliminary laboratory habituation phase the subjects were adapted to a 21-h food deprivation schedule. They were then given a period of extensive preliminary training in a problem box described by LYLE et al.[7]. A non-correction discrimination training procedure with 10 massed reinforced trials per session was employed. Test animals were permitted to make as many as 3 repetitive (perseverative) incorrect responses on any one trial and each trial terminated with a reinforced response. Training continued daily until all subjects had attained a criterion of 9 out of 10 errorless trials on each of 2 successive days. The testing of subjects from Group 1 was carried out independently of Groups 2 and 3 under slightly different conditions.

The significance of differences between groups was tested using Student's *t*-tests. The heat-stressed subjects of Group 1 made more initial (44.7 $\pm$ 12.8 ($\pm$ S.D.) vs 25.4 $\pm$ 6.1, $t18 = 4.04$, $p < 0.001$), and perseverative errors (11.3 $\pm$ 3.7 vs 7.2 $\pm$ 3.0, $t18 = 2.58$, $p < 0.02$) in the original task, and more initial errors (94.7 $\pm$ 24.9 vs 54.8 $\pm$ 8.6, $t18 = 4.32$, $p < 0.001$) and perseverative errors (17.5 $\pm$ 5.2 vs 13.0 $\pm$ 3.3, $t18 = 2.19$ $p < 0.05$) in the reversal task. They also required a greater amount of time to complete each session. The heated subjects of Group 2 made more perseverative errors than the controls in the reversal task (33.8 $\pm$ 9.6 vs 26.3 $\pm$ 5.6, $t22 = 2.53$ $p < 0.02$) but no differences were found between the performance of heated and control subjects of Group 3.

Some Group 1 offspring were also used in a simple spatial reversal learning task under similar experimental conditions. The heated subjects made more errors in the original task than control subjects (18.3 $\pm$ 8.3 and 6.4 $\pm$ 2.1, $t18 = 4.39$, $p < 0.001$) but there was no difference in the first reversal (7.0 $\pm$ 3.1 and 8.1 $\pm$ 2.5, $t18 = 0.87$, N.S.). This result suggests that differences in

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Table I. Bodyweight, age and brainweights of offspring from heated and control guinea-pigs

	Number	Body-weight (g)	Age (days)	Whole brain	Left hemisphere	Right hemisphere	Brain stem	Cerebellum
Group 1								
Control	29	600.5	173.5	4.160	1.207	1.200	1.079	0.540
$\pm$ S.D.		$\pm$109.6	$\pm$ 47.1	$\pm$0.278	$\pm$0.075	$\pm$0.089	$\pm$0.084	$\pm$0.030
Heated	31	544.0	185.1	3.304[b]	9.063[b]	0.962[b]	0.925[b]	0.416[b]
		$\pm$109.8	$\pm$ 64.5	$\pm$0.440	$\pm$0.125	$\pm$0.139	$\pm$0.120	$\pm$0.085
t-test		1.93	0.81	8.37	8.63	7.60	5.01	7.79
Group 2								
Control	12	699.3	221.9	4.366	1.268	1.287	1.192	0.560
		$\pm$118.4	$\pm$ 45.0	$\pm$0.320	$\pm$0.111	$\pm$0.109	$\pm$0.105	$\pm$0.049
Heated	21	678.4	235.4	4.141	1.257	1.252	1.093[a]	0.532
		$\pm$ 63.8	$\pm$ 28.5	$\pm$0.309	$\pm$0.073	$\pm$0.077	$\pm$0.072	$\pm$0.033
t-test		0.66	1.06	1.99	0.32	1.06	3.25	1.97

[a] $p < 0.01$. [b] $p < 0.001$ compared with group controls.

Table II. Bodyweights and brainweights of offspring from heated guinea-pigs compared with age and weight control offspring

	Age controls	t	Heated	t	Weight controls
Number of animals	6		6		6
Bodyweight (g)	630.5 [c]	6.178	449.2	0.245	441.0
± S.D.	± 44.65		± 56.38		± 59.31
Age, Days	127.2	0.374	124.7	6.705	64.8 [c]
± S.D.	± 5.34		± 15.46		± 15.46
Whole brain (g)	4.334 [c]	4.710	3.313	2.742	3.871 [a]
	± 0.1019		± 0.4630		± 0.1873
Left Hemsiphere (g)	1.200 [c]	5.208	0.930	4.557	1.168 [b]
	± 0.0519		± 0.1166		± 0.0529
Right Hemisphere (g)	1.252 [c]	5.052	0.944	3.752	1.161 [b]
	± 0.0793		± 0.1260		± 0.0648
Brainstem (g)	1.200 [b]	3.819	0.953	0.684	0.997
	± 0.0734		± 0.1410		± 0.0678
Cerebellum (g)	0.543 [a]	2.519	0.431	2.798	0.539 [a]
	± 0.0400		± 0.0830		± 0.0458

[a] $p < 0.05$. [b] $p < 0.01$. [c] $p < 0.001$ compared with heated offspring.

learning between the heated and control offspring from Group 1 were not due to visual defects.

When the behavioural trials were completed, the subjects were anaesthetized with pentobartital sodium and killed by section of the carotid arteries and jugular veins. The brains were dissected from the cranium, separated from the cord at the atlanto-axial articulation and weighed immediately to the nearest milligram. The right and left hemispheres were separated by section in the midline through the corpus callosum and each was removed from the brain-stem by section through he stria terminalis. The cerebellum was removed from the brain-stem by section of the cerebellar peduncles and each hemisphere, brain-stem and cerebellum weighed.

The whole-brain weight and weight of each brain segment of heated offspring of Group 1 females were smaller than those of control animals whereas the mean ages and bodyweight did not differ significantly. The brain-stem of offspring from mothers heated on days 40–44 was smaller than that of control animals (Table I).

As offspring of the mothers heated at 20–24 days of gestation were generally smaller than controls of the same age, a 'weight control' group of young from control mothers was selected. Each of 6 heated subjects was matched with a weight control subject of the same sex which was killed when its bodyweight was slightly less than that of its heated partner and its brain removed and weighed as before. Six randomly selected age controls are included for comparison. The results are presented in Table II. The bodyweights and weights of all brain segments of subjects from heated mothers were considerably less than weights of age controls. The weights of the whole brain, hemispheres and cerebellum of the weight control group were greater than those of the heated subjects indicating that the deficit in brain size was not merely the result of a general retardation of body growth[8].

Zusammenfassung. Trächtige Meerschweinchen wurden während verschiedenen Schwangerschaftsperioden für je 1 Stunde bei einer Umgebungstemperatur von 42–43 °C erhitzt. Ihre Jungen zeigten im nicht-räumlichen Unterscheidungstest periodenabhängige Ausfallserscheinungen. Verkleinerungen des Gesamtgehirns und der einzelnen Hirnteile als Hitzeeffekt erwiesen sich ebenfalls als periodenbezogen.

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Ein bisher nicht erkanntes Chloridzellen-Organ der Karpfenlaus *Argulus foliaceus* L. (Crustacea, Branchiura)

Argulus foliaceus besitzt auf der Ventralseite des Carapax von Kutikulaleisten begrenzte Integumentbereiche, die ursprünglich als «Schalenfelder» beschrieben und als Respirationsorgane gedeutet worden waren, weil durch den dauernden Beinschlag der Tiere ein steter Wasserstrom an ihnen vorbei geleitet wird[1]. Auch wiesen sich diese Carapaxfelder wegen der leichten Reduzierbarkeit von Schwermetallsalzen als Reduktionsorte aus, was ebenfalls auf die Atmungsfunktion hinzudeuten schien[2]. Dagegen sprach Koch[3] Organen, die sich derart mit Silbernitrat anfärben lassen, «un rôle dans la restau-

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