Enlarged Ventricles in the Cerebrum of Loop-Tail Mice

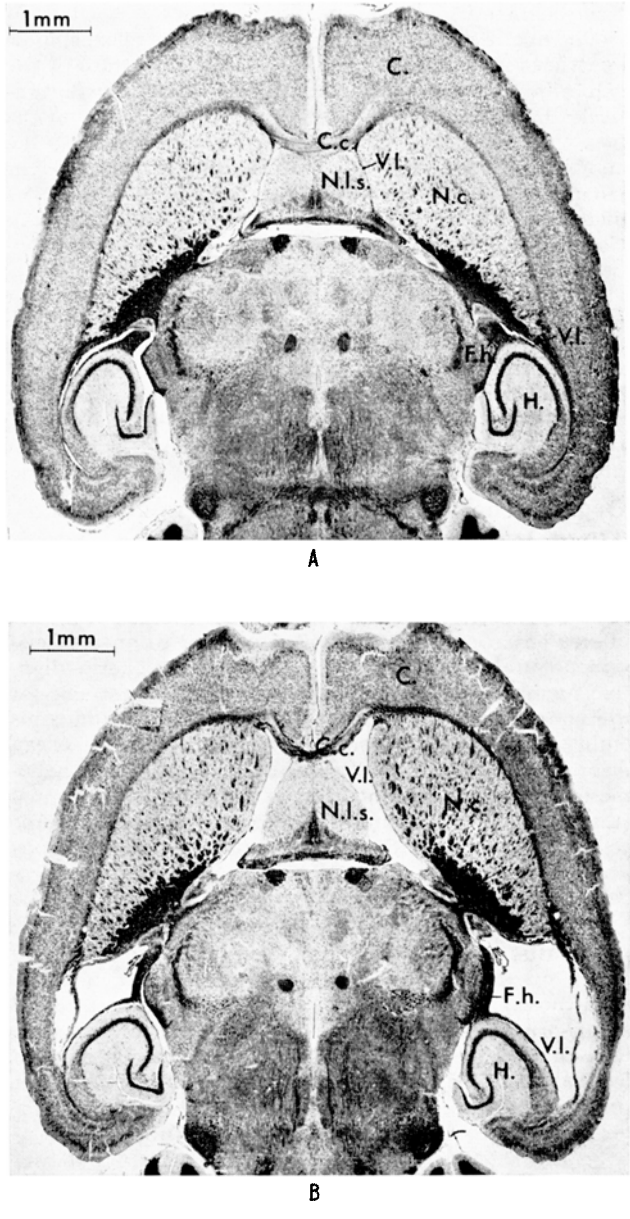
The chief problem in behaviour genetics is to clarify the mechanisms through which the genotype determines the behavioural phenotype. The study of single-gene effects, particularly in neuromuscular mutants, offers good opportunities for tracing these intermediate pathways. About 100 mutations with neurological effects are known in the house mouse[1]. The genetics and physical development of loop-tail mice have been dealt with by GRÜNEBERG[2,3]. Heterozygous animals are characterized by twisted tails and by wobbly head movements. Screening various behavioural components in adult and neonate mice, VAN ABEELEN[4,5] has observed defective motor functions in *Lp*-mutants, suggestive of impairment of extrapyramidal systems. Until now, no abnormalities in the brains of heterozygotes have been described[1]. We now present findings on structural aberrations in the telencephalon (endbrain) of heterozygous loop-tail mice, viz. enlargement of the ventricles and deformations of surrounding parts of the brain.

Experimental. Our subjects came from 2 non-inbred stocks segregating at the *Lp*-locus. Their ages varied between 5 and 15 weeks. The mutant group (*Lp*/+) comprised 4 females and 5 males, the control group 2 females and 6 males. Due to incomplete penetrance of tail twists[3], there is a chance that the control group did not consist entirely of +/+ animals. Of 3 controls, the genotypes were ascertained genetically; possibly, the remaining 5 included an unidentified mutant. We compared frontal brain sections made approximately perpendicular to the base of the skull, and horizontal sections about parallel to the base of the skull, all 10 μ thick. For this purpose, whole brains were fixed in formalin and embedded in paraffin; the sections were stained according to the method of KLÜVER and BARRERA[6].

Results. Caudal to the telencephalon, we did not detect any differences between mutants and normals. In the endbrain, however, all *Lp*-mice but 2 showed very wide lateral ventricles (sometimes only unilaterally) and a large ventriculus impar as well. All controls had normal narrow ventricles. Since the overall size of the hemispheres of these mutants appeared normal, this marked internal hydrocephalus can be expected to influence the size, form, or location of various brain regions. As is evident from the Figure (B), the caudate nucleus – putamen complex is slightly deformed at the medial margin, and the septal area, particularly the nucleus lateralis septi, is clearly malformed. The corpus callosum and the cortex seem to be reduced in some places. The hippocampal structure not only turns out to be somewhat deformed, but is also displaced in a caudal direction. Nevertheless, the connection between hippocampus and fornix remains intact, because the fimbria hippocampi is elongated. We failed to find any degeneration of nervous tissues within the affected parts of the brain.

Discussion. From the finding that 2 out of 9 *Lp*-mice did not exhibit the brain anomalies described above, it appears that penetrance of this trait is incomplete, which is also true for the tail twists and the rocking head movements. The tails of these 2 mutants were kinked, but at the time the sections were made it was unknown whether they had shown choreatic movements or not. At any rate, all mutants in which head-shaking to a marked degree was observed also had abnormal brains; the 2 traits are probably directly related.

The caudate nucleus and the putamen are involved in the regulatory functions of the extrapyramidal motor system. Therefore, the defective motor functions and the chorea found in *Lp*-mice may be traced back to the dis-



Horizontal brain sections of (A) a wild-type mouse and (B) a loop-tail mouse. V.l., Ventriculus lateralis; C., Cortex; C.c., Corpus callosum; N.l.s., Nucleus lateralis septi; N.c., Nucleus caudatus; F.h., Fimbria hippocampi; H., Hippocampus. Note wide ventricles and malformed brain structures in (B).

1 R. L. SIDMAN, M. C. GREEN and S. H. APPEL, *Catalog of the Neurological Mutants of the Mouse* (Harvard University Press, Cambridge 1965).

2 H. GRÜNEBERG, *The Genetics of the Mouse* (Nijhoff, The Hague 1952), p. 144.

3 H. GRÜNEBERG, *The Pathology of Development. A Study of Inherited Skeletal Disorders in Animals* (Blackwell, Oxford 1963), p. 153.

4 J. H. F. VAN ABEELEN, Genetica *37*, 149 (1966).

5 J. H. F. VAN ABEELEN, Anim. Behav. *16*, 1 (1968).

6 H. KLÜVER and E. BARRERA, J. Neuropath. exp. Neurol. *12*, 400 (1953).

turbed development of these parts. The rhinencephalic deformations might affect emotional determinants of their behaviour.

One might argue that the widening of the telencephalic ventricles in mutants is due to increased pressure of the cerebrospinal fluid. However, the third and fourth ventricles appeared to be unaffected by this. In view of the fact that we did not observe any proliferation of the chorioid plexuses, nor any obstructions within the ventricular system, the problem of the ultimate cause of the distension of the lateral ventricles needs further investigation.

Résumé. Les souris hétérozygotes appartenant à la mutation «loop-tail» (*Lp*) présentent une hydrocéphalie interne du télencéphale. Jusqu'ici, cette anomalie ainsi que les torsions de la queue et l'oscillation de la tête qui l'accompagnent n'avaient pas été étudiés en détail. Nous avons constaté que par suite de l'élargissement des ventricules encéphaliques, les structures cérébrales qui les entourent sont réduites, déformées ou déplacées. Ces aberrations neuro-anatomiques jettent de la lumière sur les perturbations du comportement observées chez ces mutants.

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Organogenesis: Prolonged Differentiation and Growth of Tooth Primordia on the Chick Chorio-Allantoic Membrane

The capacity for embryonic organ primordia to differentiate independently can be tested by isolation from the in situ environment and transplantation to an artificial milieu suitable for development. The chick chorio-allantoic membrane (CAM) provides a versatile host site for the maintenance and further differentiation of numerous embryonic organs[1-3]. One such organ, the tooth organ primordium, presumably arises as a result of epithelio-mesenchymal interactions. Upon entering the terminal stages of early postnatal differentiation the odontogenic cells initiate the synthesis of specific species of fibrous proteins which are involved in the formation of extracellular organic matrices.

Several investigations have indicated how rodent molar xenografts, transplanted from the host organism and grown on the CAM, advanced further developmentally than those grown in vitro[4-7]. Usually, transplantation of organ primordium on the CAM can be accomplished so that the graft is maintained for 10 continuous days of incubation. This study, however, has prolonged that period of development by culturing tooth organ primordia for many more days of uninterrupted incubation by re-transplantation to new CAM sites. The utility of the re-transplantation methodology has been successfully used and demonstrates significant potential for prolonged investigation of differentiation and growth.

Materials and methods. Donor tissues. Embryos were obtained by caesarian section from Wistar rats. The morning of the vaginal plug discovery was counted as day 0. Maxillary molar organ primordia were dissected from 19 day embryos (Figure 1). Dissection of the molar primordium was initially accomplished by procedures previously described[8]. We modified this technique to exclude any intermediate treatment of the donor tissues after observing an increased graft survival as a result of placing the excised primordium immediately on the CAM.

Controls were prepared for each litter of 19 day embryos to establish the chronological verification for the stage of development. Randomly selected maxillae were fixed and serially sectioned for this purpose.

Method of CAM-grafting. Austra White (Australop ♂ × White Leghorn ♀) embryonated hen's eggs were incubated for 8 days at 37.5 °C. On the eighth day of incubation the major branches of the vitelline blood vessels were determined by candling and appropriately recorded. The hosts were prepared for CAM grafting by procedures previously described[9]. The shell surface was cleansed with alcohol prior to cutting a 5 × 5 mm window above the region previously determined for the site of grafting. Upon elevation of the shell window, the adjacent shell membrane was incised and the underlying chorionic epithelial layer was exposed. Following explantation the shell was re-positioned and sealed with paraffin. Each host was returned to the incubator with the graft facing downward for the first 24 h of incubation; a procedure which allowed the weight of the host to facilitate the adaptation of the graft. Thereafter, each egg was turned twice daily and candled. The viability of the chick embryo was used as the criterion for assessing graft survival.

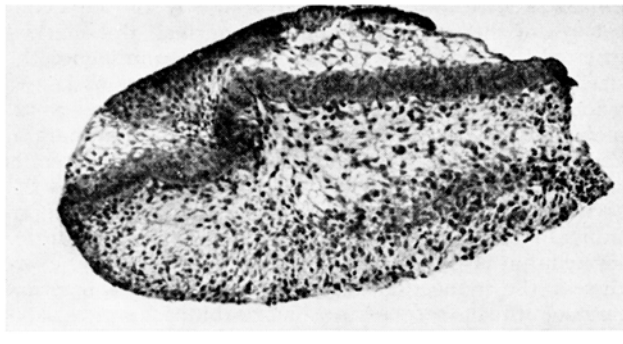

Fig. 1. The molar primordium explant immediately following excision from the donor 19-day rat embryo. Stained with hematoxylin and eosin. × 65.

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