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Abnormal development of the facial nerve nucleus in reeler mutant mice

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INTRODUCTION

Reeler (rl) mice, an autosomal recessive mutation (Falconer, 1951), sustain an abnormal development of cortical structures, which have been extensively studied (Caviness & Rakic, 1978). It has also been shown that the reeler gene affects the cochlear nucleus (Martin, 1981), the inferior olivary complex (Goffinet, 1981), the tectum and the lateral geniculate nucleus (Frost, Caviness & Sachs, 1982).

The detection of new targets of the reeler mutation and developmental studies of the reeler phenotype at various sites should provide further clues as to the action of this mutant gene. In addition, the widespread effect of the reeler gene on the developing nervous system accentuates the importance of that locus during normal embryogenesis.

The present paper describes the comparative architectonics and development of the facial nerve nucleus in normal and reeler mice. The facial nucleus, as well as other rhombencephalic structures (lateral reticular nucleus, trigeminal complex), are newly recognised targets of the reeler gene.

MATERIALS AND METHODS

Animals

The Orléans allele of the reeler mutation (rl^{Orl}), originally provided by Guenet (Institut Pasteur, Paris), was maintained on a recombinant BALB/c × 129/Sv strain. Pregnancies were dated by checking for vaginal plugs; embryonic day zero (E0) corresponded to the first day on which a plug was found. Before E13–E14, the reeler phenotype was not distinct; reeler embryos at E11 and E12 were obtained by mating homozygous animals.

Histology

Embryos were fixed by immersion of the whole body (until E15) or the head (from E16 onwards) in Bouin Hollande fixative, and embedded in paraplast; serial sections 7 or 10 μm thick were cut and stained with haematoxylin and eosin.

Adult brains were fixed by intracardiac perfusion of ether anaesthetised animals, under gravity flow, using 10% formalin in 0·1 M phosphate buffer, pH 7·4.

Autoradiography

Autoradiographic determination of the last cell division ('neuronal birth dates') was performed according to Sidman (1970).

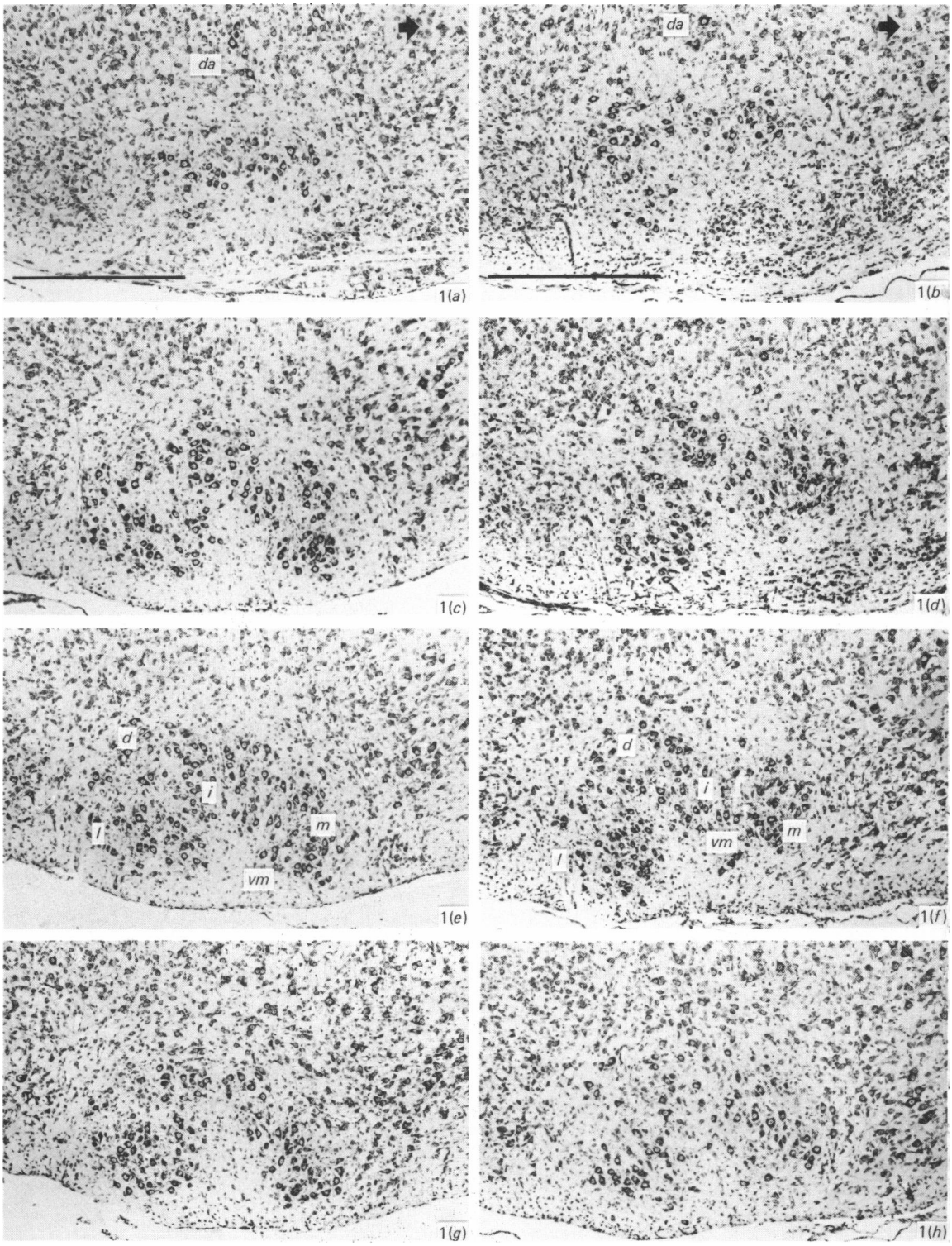


Fig. 1 (*a–h*). Photomicrographs of the facial nucleus in normal (left) and reeler (right) adult mice, at four levels from rostral (above) to caudal. The arrows point towards the raphe. The lateral (*l*), medial (*m*), ventromedial (*vm*), intermediate (*i*) and dorsal (*d*) divisions are clearly visible. *da*, dorsal accessory nucleus; bar, 500 μm.

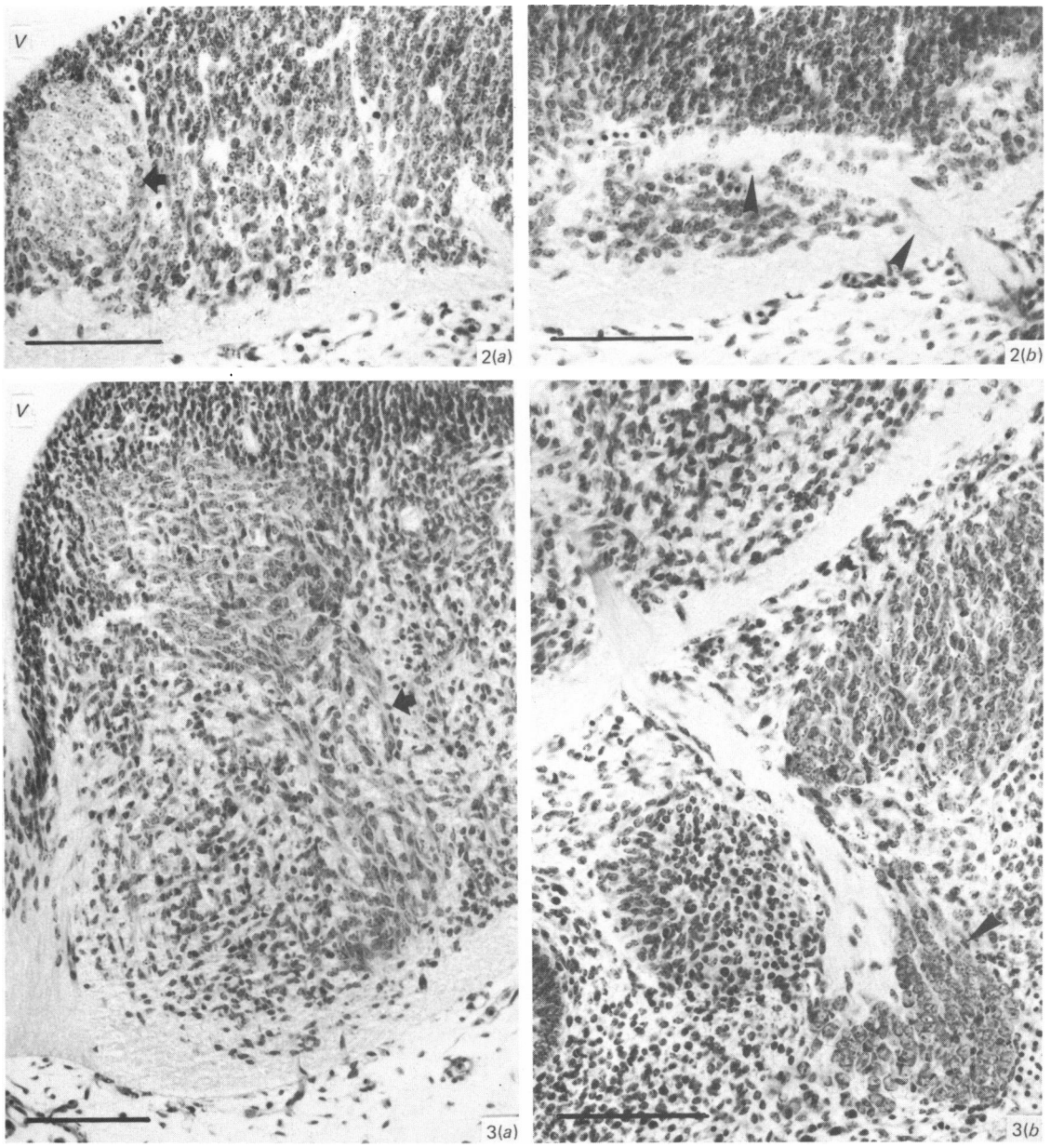


Fig. 2 (*a–b*). The early facial nucleus (*a*, arrow) and facial nerve (*b*, arrowheads) are clearly visible at E11. *V*, fourth ventricle; bar, 100 μm.

Fig. 3 (*a–b*). At E12, the neurons of the facial nucleus are actively engaged in radial migration (*a*, arrow). The facial nerve contains sensory fibres coming from the facial ganglion (*b*, arrowhead). *V*, fourth ventricle; bar, 100 μm.

Cell counts

The neurons of the facial nuclei were counted in serial paraffin sections 10 μm thick. Only nucleoli were counted, and corrections for double counting were made according to Abercrombie (1946).

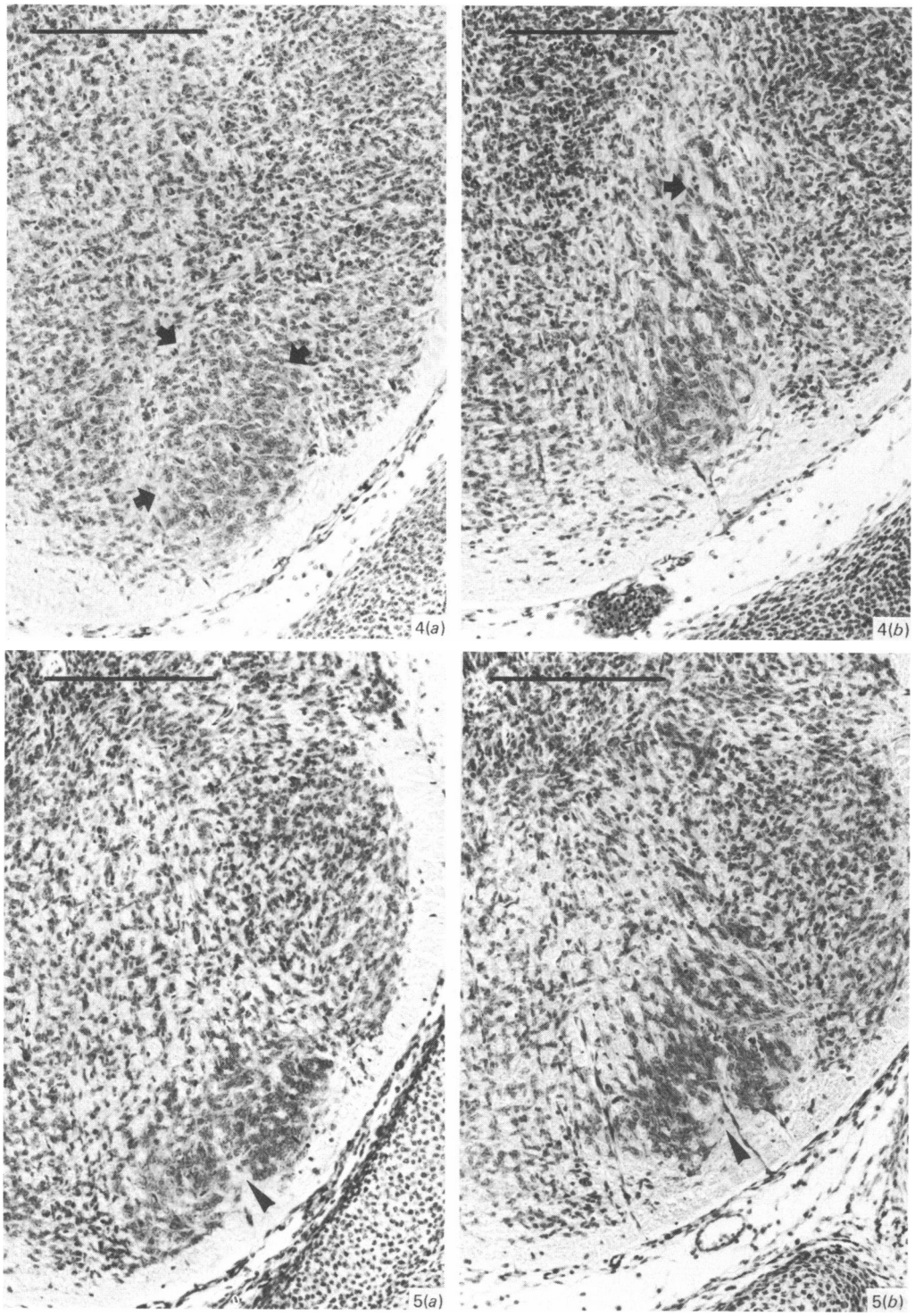


Fig. 4 (*a–b*). At E13, the normal facial nucleus is rounded (*a*, arrows), whereas several rows of neurons extend radially in the parenchyma in mutant embryos (*b*, arrow); bar, 200 μm.

Fig. 5 (*a–b*). At E14, the normal (*a*) and reeler (*b*) facial nuclei differ in density and definition. The subdivision in two masses occurs in both genotypes (arrowheads); bar, 200 μm.

RESULTS

Comparative architectonic analysis (Fig. 1)

In normal mice, the facial nucleus was located near the ventrolateral surface of the medulla oblongata, extending rostrocaudally over approximately 560 μm. Its neuronal population was estimated (in two nuclei) to be 2186 cells. As seen in sequential coronal sections, fibre bundles appeared to divide the facial nucleus into at least five divisions: medial, ventromedial, intermediate, lateral and dorsal divisions (Fig. 1*e*; nomenclature of Martin & Lodge, 1977). A small neuronal cluster was located rostrally, dorsal to the main nucleus, and corresponded to the dorsal accessory (Ashwell, 1982) or suprafacial nucleus (Watson, Sakai & Armstrong, 1982). The medial division was distinct from other components throughout the length of the nucleus, and extended slightly more rostrally than other divisions. The lateral and intermediate divisions were not clearly separated rostrally. Similarly, the dorsal and ventromedial divisions were well defined only in the median portion of the nucleus. At the most caudal level, only the lateral division was found. In some sections, the definition of small components was difficult, as minor cell clusters were scattered between the main divisions.

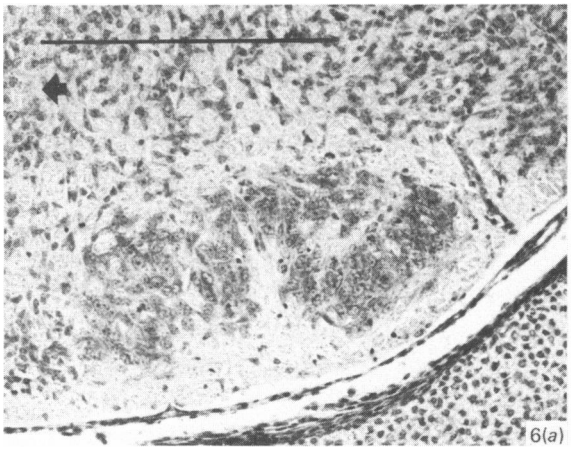
In reeler mutant mice, the facial nucleus was usually located near the ventrolateral surface of the pons, and extended rostrocaudally over approximately 590 μm. The neuronal contingent of the reeler nucleus was estimated (in two nuclei) to be 2070 cells. A small dorsal accessory nucleus was present rostrally, but lay deeper in the parenchyma than in the normal animal. In coronal sections, the main body of the facial nucleus was wedge shaped and spread out more widely in a radial direction than its normal counterpart. The divisions of the mutant nucleus had the normal topographical relationships (Fig. 1*f*), but as a rule were less clearly defined than in the normal nucleus. The medial and lateral divisions were clearly visible. By contrast, it was difficult to delimit the dorsal, ventromedial and intermediate components, as their neurons were scattered or grouped in small clusters, without distinct architectonic features.

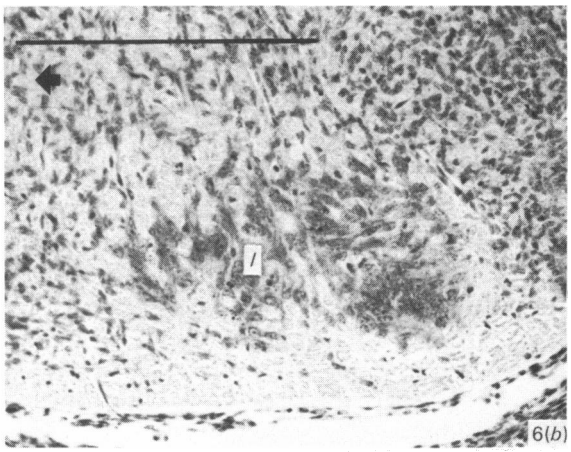
Embryological analysis (Figs. 2–7)

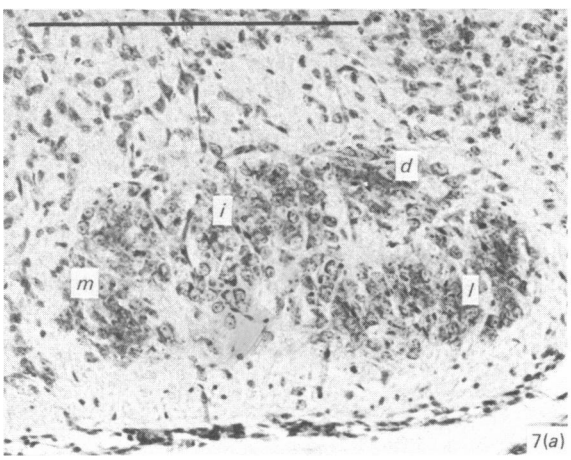
The embryonic development of the facial nucleus was examined from embryonic day eleven (E11), when its cells could be morphologically identified.

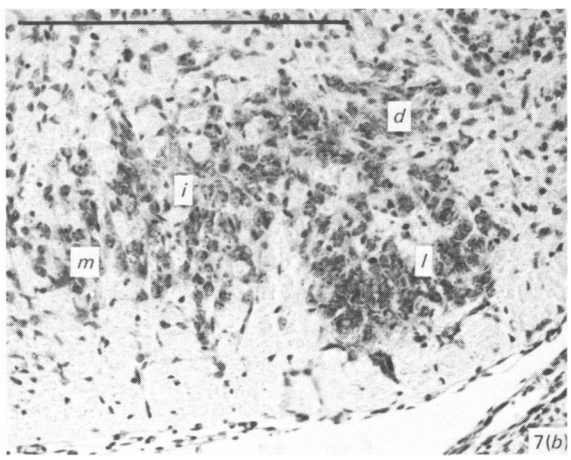
At E11 and E12, no difference was observed between reeler and normal embryos. At E11, the facial nucleus was located medially, near the fourth ventricle, and had a rounded shape (Fig. 2*a*). Its immature neuronal cells had globular nuclei (6–7 μm in diameter) with pale chromatin, and a sparse cytoplasm. Axons of these early neurons formed a bundle which ran laterally across the parenchyma to emerge from the lateral surface of the medulla. This bundle also contained sensory fibres from the facial ganglion, and corresponded to the early facial nerve (Fig. 2*b*). At E12 (Fig. 3*a*, *b*), the immature facial neurons were radially elongated, and contained clear, ellipsoidal nuclei (approximately 13 by 7 μm), with their cytoplasm distributed at either end of the cell. They were arranged in radial columns extending from the germinative zones to more superficial levels. The direction of migration was parallel to the processes of neuroepithelial cells and to penetrating blood vessels. At the same time as radial neuronal migration proceeded, a bend developed in the intraparenchymatous course of the facial nerve.

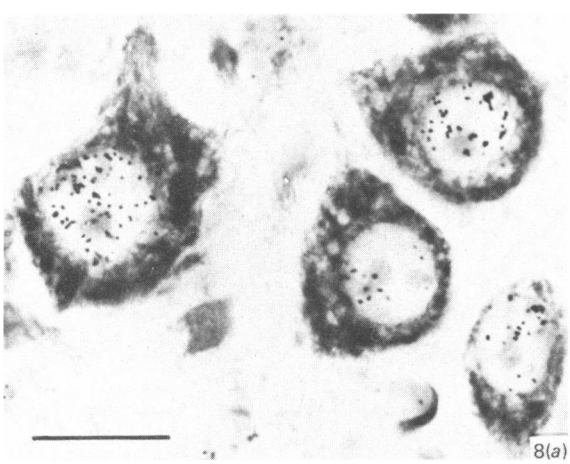
At E13 (Fig. 4*a*, *b*), the migration of facial neurons continued, and was nearly completed at E14 (Fig. 5*a*, *b*). At that stage, the reeler facial nucleus was for the first











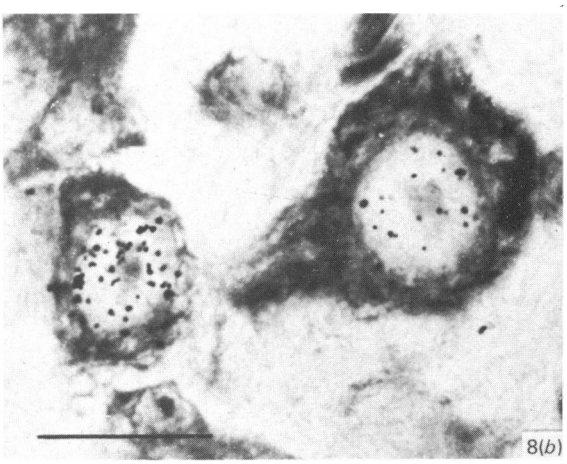


Fig. 6(*a–b*). At E15, the major divisions begin to form both in normal (*a*) and reeler (*b*) embryos. The arrows point towards the mid-line. *l*, lateral division; bar, 300 μm.

Fig. 7(*a–b*). At E17, the principal divisions are identified in the normal nucleus (*a*). In the mutant nucleus (*b*) they seem to be present but are less distinct than in normal animals. *d*, dorsal; *i*, intermediate; *l*, lateral; *m*, medial division; bar, 300 μm.

Fig. 8(*a–b*). Labelled neurons are found in normal (*a*) and reeler (*b*) facial nuclei, when the embryos received a thymidine pulse at E10; bar, 40 μm.

time different from its normal counterpart. In normal fetuses, the superficially located nucleus appeared as a well defined, rounded neuronal mass (Figs. 4*a*, 5*a*). The nucleus was beginning to undergo subdivision, and lateral and medial masses were visible. In reeler animals, the facial nucleus lay slightly deeper in the parenchyma and was less distinct from surrounding structures than in normal fetuses (Figs. 4*b*, 5*b*). Several neurons were positioned heterotopically in the depth of the tissue. Division into medial and lateral components was also seen in the mutant nucleus, for the first time, at E14.

After completion of neuronal migration, the fragmentation of the nucleus into its various divisions proceeded rapidly. The major components were clearly visible at E15 (Fig. 6*a*, *b*). By E17 (Fig. 7*a*, *b*), the maturation of the nucleus was nearly achieved and, with the exception of minor cell components like the ventromedial division, the architectonic pattern was similar to that of the mature nucleus. In the mutant embryo, the chronology of maturation in the facial nucleus appeared normal. In addition, there were the same divisions as in the normal animal and their relative topography was conserved. However, the definition of the various components from each other, from surrounding structures and from fibre strata, was less distinct than in the normal nucleus.

Autoradiographic analysis (Fig. 8*a*, *b*)

No neuronal cell was labelled in the facial nuclei of mice which had received a [^{3}H]thymidine pulse at E11–E14. By contrast, several neurons were labelled when the thymidine was administered at E10. The intensity and pattern of labelling were similar in the nuclei of reeler and normal litter mates.

DISCUSSION

Origin and proliferation of facial neurons

The autoradiographic observations show that several facial neurons are generated at E10, in both genotypes. Some of them could be generated earlier at E9. This stage, which requires intraembryonic injection of the tracer (Sidman, 1970), has not been investigated. The 'birth date' of facial neurons has been determined in normal mice (Taber, 1964), with essentially the same result. In the rat, Altman & Bayer (1980) have demonstrated that the majority of neurons in the facial nucleus are generated at E13. In the present study, the number of neuronal cells in the facial nucleus of normal and reeler mutant mice has been estimated respectively at 2186 and 2070 cells. Counting is slightly more difficult and less accurate in mutant nuclei, due to their poor definition. These numbers compare well with those of Ashwell (1982) and show that the reeler mutation does not result in atrophy of the nucleus.

Cell migration and the early facial nerve

The formation of the facial nerve occurs very early, at E11, about 24 hours after facial neurons are generated and before cell bodies begin their radial migration at E12. An analogous observation has been made recently in rat embryos by Altman & Bayer (1982). The facial nerve also contains fibres from the facial ganglion, but the data do not reveal which contingent of fibres, if any, is the first to enter the nerve.

In contrast to axonal growth, which proceeds laterally in the facial nerve, the migration of neuronal cell bodies is radial, parallel to the direction of neuroepithelial cells and of penetrating blood vessels. The mechanism responsible for the

radial migration of facial neurons remains unknown, and the principle of neurobiotaxis (Kappers, Huber & Crosby, 1936) can hardly account for it (Altman & Bayer, 1982). A guiding factor might be provided by radial extensions from neuroepithelial cells. It has been amply demonstrated that radial glial fibres act as guides for radial neuronal migration in the cerebral cortex and cerebellum (Caviness & Rakic, 1978; Pinto-Lord, Evrard & Caviness, 1982).

The architectonics of the facial nerve nucleus

After the completion of neuronal migration, the maturation of the facial nucleus proceeds rapidly to the adult pattern. The architectonics of the facial nucleus have been described in the rat by Papez (1927) and by Hogg (1928). Recently, a correspondence between the subdivision of the nucleus and its musculotopic organisation has been demonstrated in rats (Martin & Lodge, 1977; Watson *et al.* 1982) and in mice (Ashwell, 1982). The observation of a nearly normal pattern of division in the reeler facial nucleus suggests that a musculotopic representation of facial muscles is also present in the mutant nucleus.

The facial nucleus and the reeler mutation

In addition to containing a normal contingent of neurons, the facial nucleus in reeler mutant mice develops according to a normal schedule into topologically well organised subdivisions. The reeler phenotype becomes identifiable at the end of neuronal migration. There is an abnormal formation of the early architectonics of the nucleus, while the mechanisms of directional axonal growth appear unaffected.

In all these respects, the developmental defect of the facial nucleus in reeler mutants is similar to that described in the cerebral cortex (Pinto-Lord & Caviness, 1979; Goffinet, 1979), the inferior olive (Goffinet, 1981), and the cerebellum (Caviness & Rakic, 1978). It is tempting to suggest that the same mechanism accounts for the pleiotropic effect of the gene, namely an abnormal interaction between neurons and radial fibres (Pinto-Lord *et al.* 1982) and/or between neurons themselves (Goffinet, 1979) and the end of radial migration.

SUMMARY

The architectonics and development of the facial nerve nucleus have been compared in reeler and normal mice. In both genotypes neurons were generated at E10, and their axons entered the facial nerve at E11. Cell bodies migrated radially at E12–E13, possibly along radial neuroepithelial fibres. From the end of migration, the reeler nucleus was less superficially located and less clearly organised than the normal nucleus. The normal adult architectonic divisions were present in the mutant, but less distinct than in the normal nucleus. These observations have been related to other data on the developmental biology of the reeler brain. It is suggested that the reeler gene could affect an interaction between neurons or between neurons and radial fibres at the end of migration.

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