USE OF LINKAGE AND CYTOLOGICAL MARKERS TO IDENTIFY MICE DOUBLY AFFECTED BY "STAGGERER" AND "REELER" CONDITIONS

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STAGGERER" (SIDMAN, LANE and DICKIE 1962) and "reeler" (FALCONER 1951; HAMBURGH 1963) are neurological mutations in mice, both of which cause ataxia. They are both recessive and not linked with each other (GREEN and LANE 1967). The behavior of the two mutants is not identical but it is virtually impossible to distinguish with any confidence one type from another by the observation of behavior alone.

Both mutants show extensive alterations in the cytoarchitectonics of the cerebellum (HAMBURGH 1963; SAX, HIRANO and SHOFFER 1968; SIDMAN *et al.* 1962; SIDMAN 1968). The size of the cerebellum is grossly reduced and there are derangements of various cell populations. Reeler mice show additional changes in the cerebrum, including an abnormal hippocampus and fascia dentata.

In order to determine the combined effect of the two mutant genes, staggerer and reeler, upon viability, behavior and cytoarchitectonics of the cerebellum, a method was devised to produce mice doubly affected by these conditions and to identify them with accuracy.

MATERIALS AND METHODS

One of the difficulties for the study of mice affected by both the staggerer and reeler conditions lies in the fact that males affected by either condition never breed, although females may occasionally breed. This necessitated the use of heterozygotes for the production of both the F_1 and F_2 generations.

Since staggerer mice cannot be distinguished from reeler mice by the observation of behavior alone, efforts were made to obtain the staggerer and reeler genes with suitable genetic markers. Staggerer (*sg*) is closely linked with dilution (*d*) and short ear (*se*) with a recombination frequency of 4.49% (GREEN and LANE 1967). Therefore, *d* and *se*, which rarely show recombination, were used to mark the staggerer condition. Although the luxate gene (*lx*) is known to be linked with reeler (*rl*) (FALCONER 1952), the linkage was too loose to be useful. Instead, cytoarchitectural alterations in the hippocampus and fascia dentata of cerebrum were used to distinguish the reeler condition. Excellent photographs of these structures in reeler mice and their normal litter mates have previously been published (HAMBURGH 1963). Reeler mice can easily be distinguished from their normal litter mates by the appearance of these structures.

The staggerer strain was descended from a pair of mice obtained from the Jackson Laboratory in 1965 and it has since been maintained by brother-sister matings. This strain carried *d* and *se* in addition to *sg*. From this strain, *d se sg/+ + +* mice were selected to mate with mice heterozygous for *rl*. The reeler strain also originated from a pair of mice obtained from the same laboratory in 1966 and has been maintained by brother-sister matings. Offspring from these

matings, which were designated as F_1 , were then mated to each other to produce double mutants among F_2 mice.

Only about $\frac{1}{4}$ of F_1 matings are expected to produce dilute mice with short ears. Therefore, F_1 matings which produced no dilute mice with short ears in the first 2 to 3 litters were discarded, since these matings were not suitable for producing staggerer mice with the dilution and short-ear markers. This process quickly eliminated 35 of a total of 45 F_1 matings, leaving only 10 matings to be examined.

Litters from these 10 matings were killed at age 2 weeks or later and histological sections were prepared from the brains of mice that showed the neurological disorder, hereafter called neurological mutants. Eight of these 10 F_1 matings produced no neurological mutants that had abnormal hippocampus and fascia dentata, which is a characteristic of reeler mice. Therefore, these 8 matings were subsequently discarded, since they were not suitable for producing reeler mice. This left only two F_1 matings. Each of these two matings produced two types of neurological mutants: those with normal hippocampus and fascia dentata and those with altered hippocampus and fascia dentata, identical to the reeler cerebrum. Accordingly these two matings were assumed to be of the following type (hereafter called double heterozygote mating):

$$rl/+; d\ se\ sg/+ \times rl/+; d\ se\ sg/+$$

In the absence of recombination, non-dilute normal-eared neurological mutants from these matings should be reelers and dilute short-eared neurological mutants should be either staggerers or double mutants. Therefore, the search for double mutants were made among those with dilution and short ears.

In order to prepare histological sections, brains were dissected out and frozen in a cryostat. Sagittal sections were cut 6μ thick and were stained with hematoxylin and eosin.

RESULTS

The two double heterozygote matings described in MATERIALS AND METHODS produced 128 F_2 mice. Twenty-six of these had dilution and short ears and they all showed neurological disorder. Of these 26, 20 had a normal hippocampus and fascia dentata, indicating that they were staggerer mice. The remaining 6 mice had an altered hippocampus and fascia dentata and accordingly they were considered to be affected by the reeler condition. Whether these 6 mice were affected by the staggerer condition also is discussed below.

Since the recombination frequency between *d se* and *sg* is 4.49%, it can be shown that 91.4% of the 6 reeler mice that had dilution and short ears are expected to be staggerers. Accordingly it was concluded that at least some of these 6 mice were double mutants, affected by the staggerer and reeler conditions. Statistically it is possible that all these 6 mice were double mutants and this is shown in Table 1. Also the χ^2 value shown in the Table rules out the possibility that double mutants are not viable.

There were 30 neurological mutants that had non-dilution and normal ears among the offspring from the two matings. A histological examination was carried out with 12 of these mice. As expected, all but one mouse showed altered hippocampus and fascia dentata, and therefore they must have been reelers. The cytoarchitectonics of the cerebellum of one exceptional mouse was of a typical staggerer type and therefore this mouse was considered to be a recombinant.

The cytoarchitectonics of the cerebellum of the 6 mutants shown to be possible double mutants was different from that of either of the two single mutants, although the general outline resembled that of the reeler mice more than that of

TABLE 1

Expected and observed number of various types of neurological mutants with dilution and short ears from matings between mice of genotype, $rl/+$, $d\ se\ sg/+ +$

Bases of computations	Types of mutants			Total	χ^2	P
	<i>d se sg</i>	<i>d se rl</i>	<i>d se sg rl</i>			
Expected percentages of 3 types on the basis of 4.49% recombination between <i>d se</i> and <i>sg</i>	73.28	2.30	24.42	100	...	...
Expected numbers of 3 types on the basis of above percentages	19.05	0.60	6.35	26	...	...
Observed numbers of 2 types: neurological mutants with dilution and altered hippocampus considered as double mutants	20	...	6	26	0.69	>0.05
Expected percentages of 2 types, if double mutants are assumed to be not viable	96.96	3.04	...	100	...	...
Expected numbers of 2 types on the basis of above percentages	25.21	0.79	...	26	...	...
Observed numbers of 2 types, with assumption that double mutants are lethal and they are considered as reeler mice	20	6	...	26	35.44	<0.001

the staggerer mice. The size of the median sagittal sections of the cerebellum of these mutants was significantly smaller than that of the staggerer or reeler cerebellum. (Median sagittal sections of cerebellums of staggerer and reeler mice of comparable age are approximately equal in size.) It ranged, at 12 to 14 days of age, from 1/2 to 1/3 of that of the single mutant, which is itself reduced to 1/2 to 1/3 of that of the normal litter mate. The size of the same section of these mutants, at 17 to 19 days of age, ranged from 1/3 to 1/4 of that of the single mutant. This is shown in Figure 1.

The molecular layer of the staggerer and reeler cerebella is significantly thinner than that of their normal litter mates. In the cerebella of these 6 mutants, this layer was almost obliterated. The fourth ventricle at the median sagittal section is much larger than the normal in both staggerer and reeler cerebella, but in these 6 mutants this structure was further enlarged and exceeded the size of the cerebellum itself. Thus, the cytoarchitectonics of these 6 mutants differed significantly from that of either staggerer or reeler. Accordingly it was concluded that these 6 mutants were all double mutants and that the effects of these two sets of mutant genes upon the cytoarchitectonics of the cerebellum were additive in many respects.

The behavior of the double mutants was similar to that of staggerer and reeler mice. Thus, no classification of double mutants was possible from observation of behavior alone.

FIGURE 1.—Median sagittal section of the cerebellum of reeler (A), double mutant (B) and normal mouse (C). The outline of the cerebellum is indicated by an arrow. m, molecular layer; g, internal granular layer; v, the fourth ventricle. The molecular and granular layers of the double mutant are not labelled. Age, 19 days. Magnification, about 36 X (A and B) and about 20 X (C).

These results point to the possibility of producing additional double mutants affected by various combinations of neurological genes that affect the cytoarchitectonics of the mouse cerebellum, and of accurate classification of these double mutants even in the absence of suitable genetic markers. Studies of these double mutants should provide valuable information for the elucidation of mechanism of morphogenesis of the mouse brain.

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SUMMARY

The use of linkage and cytological markers in the production and identification of neurological mutants doubly affected by staggerer and reeler conditions is discussed. Double mutants were indistinguishable from single mutants in their behavior but showed alterations in the cytoarchitectonics of the cerebellum, different from those found in the cerebellum of single mutants.

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