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The probable mode of gene action in the circling mutants of the mouse

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1. INTRODUCTION

Mutations producing an abnormality of behaviour commonly known as the waltzing syndrome are remarkably frequent in the mouse. About thirty such genes are already known (Sidman, Green & Appel, 1965), and this number is rapidly increasing. The distinguishing feature of affected animals is a tendency to run in circles, which is usually accompanied by deafness, jerking movements of the head, hyperactivity, impairment of the ability to swim, tilting of the head and abnormal responses to linear and angular accelerations. Not every mutation produces all of these traits, and not all animals carrying a particular gene necessarily have the same behaviour, but the mutants have enough in common to form a readily identifiable group. The majority of these mutants have been the subjects of detailed anatomical, and where necessary embryological, studies. In all cases abnormalities of the inner ear have come to light. The abnormalities may be degenerative, with breakdown of certain structures following normal differentiation, or morphogenetic, with differentiation of the entire ear or a part of it taking place along faulty lines. This distinction is by no means strict, but it is useful in the discussion of these mutants.

Since the inner ear is the seat of the sense of equilibrium, and this sense appears to be disturbed in these mutants, there has been a tendency to assume that the defects of the inner ear are responsible for the abnormal behaviour, particularly when gross defects of the semicircular ducts occur (Lyon, 1960; Stein & Huber, 1960; for otological terms used see Bast & Anson, 1949). However, a close examination of the published information showed that such an assumption was hard to maintain. On the contrary, there were strong indications that the abnormalities of behaviour were not consequent on those of the inner ear. First, extirpation of the whole or a part of the inner ear, whether unilateral or bilateral, does not lead to circling (Löwenstein, 1936; Prosser, 1950). Secondly, in artificial waltzers, produced by means of drugs, no abnormalities of the inner ear could be detected, although widespread lesions were observed in the cerebellum and brain stem (Goldin, 1947; Goldin, Noe, Landing, Shapiro & Goldberg, 1948). Thirdly, fundamental anatomical differences may be observed in mutants with indistinguishable behaviour.

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Fourthly, with the exception of the abnormalities of the cochlea and deafness, no particular defect of the inner ear is invariably associated with any specific peculiarity of behaviour.

The last two points need elaboration. In the mutant shaker-1 no abnormalities of the inner ear have been observed before the age of about three weeks (Grüneberg, Hallpike & Ledoux, 1940; Deol, 1956), whereas the abnormal behaviour can often be detected even at the age of one week. Moreover, the behaviour of shaker-1 mice is almost identical with that of kreisler (Hertwig, 1942, 1944), in which the inner ear is so grossly malformed that it does not bear much resemblance to the normal organ (Deol, 1964*a*). The only common pathological factor among the mutants jerker, shaker-1, shaker-2, pirouette, spinner, varitint-waddler and waltzer is the degeneration of the macula of the saccule and the organ of Corti (Deol, 1954, 1956; Deol & Robins, 1962), but these structures are reported to be normal in fidget (Truslove, 1956), twirler (Lyon, 1958), waltzer-type (Stein & Huber, 1960) and zigzag (Lyon, 1960). On the other hand, in the latter group gross defects of one or more semicircular ducts are always present, but not in the former. Further, while all waltzer-type mice have some defect of the semicircular ducts, many of them do not show any abnormality of behaviour (Stein & Huber, 1960). Only defects of the lateral semicircular duct were found to be associated with abnormal behaviour, but even to this there were a number of important exceptions. The same applies to the mutant zigzag, in which also the lateral duct is affected (Lyon, 1960). The only mutant in which a peculiarity of behaviour and a unique type of defect of the inner ear go together is fidget. These mice shake their heads in the horizontal plane and lack the crista of the lateral duct (Grüneberg, 1943; Truslove, 1956). But even here one should be careful not to assume a causal relationship between the two features. In the textbook view of the mode of function of the crista the related semicircular duct plays an essential part. Consequently the absence of the lateral crista should have the same effect as the absence of the lateral duct or its closure anywhere along its arc. This happens in the mutant zigzag in which there is no head jerking at all (Lyon, 1960), and in waltzer-type in which the head is moved in the vertical plane instead of the horizontal (Stein & Huber, 1960). In any case the absence of the lateral crista in fidget mice is little more than a supererogatory defect, for the related duct itself is missing.

It may be thought that perhaps it is not the degeneration or the malformations of the inner ear that are responsible for the abnormal behaviour, but anomalous function of the maculae and cristae. No extirpation experiments on circling mice have been performed, but there is evidence that this is probably not the case. If it were, there should be some improvement in the condition of kreisler and Snell's waltzer mice in old age as a result of the degeneration of almost the entire neural epithelium in the inner ear (Deol, 1964*a*; Deol & Green, unpublished). No such change has been observed.

It appeared from these considerations that the attribution of abnormal behaviour of circling mice to defects of the inner ear was probably without foundation. However, it was considered desirable to verify this by anatomical studies on animals

with different types of behaviour abnormalities, and to see whether any of the inconstant features of the behaviour syndrome, such as impairment of the ability to swim, is always associated with some anatomical peculiarity. The most suitable mutant for this purpose seemed to be kinky (symbol Fu^{ki}), for a preliminary survey had revealed that it had the widest range of ear defects of all the mutants, combined with behaviour that varied from normal to the full manifestation of the waltzing syndrome, with many intergrades. Although it has long been known that in addition to the malformations of the axial skeleton, after which the gene is named, kinky mice often show abnormal behaviour and deafness (Caspari & David, 1940; Dunn & Caspari, 1945), the inner ear of these animals has not been examined before.

2. MATERIALS AND METHODS

Thirty mice of the $Fu^{ki}/+$ genotype, varying in age from 26 to 459 days, were selected to get a wide range of abnormalities of behaviour. They were fixed in Witmaack's fluid, and embedded first in celloidin and then in paraffin. Serial sections of the otic capsule along with the adjacent part of the brain were cut at 10 μ in a plane parallel to the modiolus in the cochlea, and stained with haematoxylin and Orange G containing a trace of erythrosin. The details of the behaviour of these mice are given in Table 1. Deafness, occurring in circlers and non-circlers alike, has been left out, because it showed complete correlation with defects of the organ of Corti, which were always of the morphogenetic type. Swimming ability was tested in two ways: by dropping the animals into water from a height of approximately 6 in., and by placing them gently on the surface of the water. Swimming ability was regarded as lacking when an animal failed to stay close to the surface, even momentarily, in both tests, and would have drowned if not rescued quickly. It was classified as poor when an animal succeeded, at least for a few seconds, in staying on the surface when put there gently, but would have failed to survive without assistance when dropped into the water. Swimming ability was considered normal when the animal was able to keep itself from drowning in both cases. This elaborate description of a simple test is necessary because different authors appear to mean different things by swimming ability. The animals were easy to classify for circling, jerking movements of the head and hyperactivity, particularly on being gently prodded a few times. Tilting of the head, when present, was always a permanent condition, unlike a similar abnormality observed in the pallid mutant (Lyon, 1951, 1953). Its extent varied from a slight departure from the normal position to a state where one of the ears almost touched the floor.

3. OBSERVATIONS

The abnormalities found in the inner ear are set out in Table 1. Virtually all of them were of the morphogenetic type. Some degeneration of cells in the neural epithelium was occasionally observed, but it was never quite reminiscent of the mutants of the degenerative group. The abnormalities of the saccule and utricle were roughly of the same kind as in the mutant shaker-with syndactylism (Deol,

Table 1. *Anomalies of behaviour and malformations of the inner ear in 30 mice of* $Fu^{ki}/+$ *genotype*

Number	Reference number	Age in days	Circling	Head jerking	Hyperactivity	Swimming ability	Head tilting	Saccule	Utricle	Semicircular ducts
1	593	26	+	+	+	–	–	A/A	N/A	N/A
2	594	41	+	+	+	–	R	A/A	N/N	N/A
3	776	273	+	+	+	–	R	N/N	N/N	N/N
4	780	90	+	+	+	–	–	A/A	N/N	A/A
5	819	90	+	+	+	–	R	A/A	N/N	A/A
6	820	120	+	+	+	–	R	A/A	N/N	N/N
7	821	28	+	+	+	–	–	A/A	A/N	A/A
8	943	242	–	–	+	–	R	N/A	N/A	N/A
9	948	228	–	–	+	–	R	N/A	N/A	N/A
10	777	90	–	–	–	P	R	N/A	N/A	N/A
11	778	120	–	–	–	P	L	A/N	N/N	N/N
12	870	415	–	–	–	P	R	A/A	A/N	A/N
13	871	434	–	–	–	P	L	A/A	A/N	A/N
14	940	221	–	–	–	P	R	N/N	N/N	N/N
15	941	221	–	–	–	P	L	N/A	N/N	N/N
16	942	221	–	–	–	P	R	N/A	N/N	N/A
17	944	156	–	–	–	P	–	N/N	N/N	N/N
18	945	156	–	–	–	P	–	N/N	N/N	N/N
19	946	346	–	–	–	P	R	A/N	A/N	A/N
20	947	202	–	–	–	P	R	N/N	N/N	N/N
21	950	459	–	–	–	P	R	N/A	N/A	N/A
22	951	226	–	–	–	P	L	A/N	A/N	A/N
23	644	408	–	–	–	N	–	N/N	N/N	N/N
24	789	120	–	–	–	N	–	N/N	N/N	N/N
25	838	93	–	–	–	N	–	N/N	N/N	N/N
26	839	127	–	–	–	N	–	N/N	N/N	N/N
27	840	193	–	–	–	N	–	A/N	N/N	N/N
28	861	134	–	–	–	N	–	N/N	N/N	N/N
29	862	134	–	–	–	N	L	N/N	N/N	N/N
30	949	128	–	–	–	N	–	A/A	N/N	N/N

Explanation of symbols. Under circling, head jerking and hyperactivity: + = present, – = absent. Under swimming ability: – = lacking, P = poor, N = normal. Under head tilting: – = position of head normal, L = left side tilted downwards, R = right side tilted downwards. Under saccule, utricle and semicircular ducts: N = normal, A = abnormal; the two sides being given as Left/Right.

1963). The macula was in contact with the free wall, and the cavity virtually obliterated. The otolith membrane or its equivalent gelatinous mass was broken up by sheets of undifferentiated cells, and the otoliths scattered or reduced or even missing. The semicircular ducts, when affected, were either short and extremely wide, or narrow and occluded in places. The cristae were poorly differentiated, so

that the orderly arrangement of the neural epithelium and the cells of the supporting layer was lacking. In setting out the abnormalities of the semicircular ducts all three ducts have been treated as a unit, because as a rule either all of them were affected or none. In the rare cases where only one or two were affected the whole unit was classified as abnormal, for no advantage was seen in doing otherwise. Nor was any distinction made between the abnormalities of the semicircular ducts and the cristae, because the resulting interference with function would be much the same in both cases. In the few instances where a part of the macula of the saccule or utricle appeared normal the classification is of necessity subjective, for it had to be decided whether normal function was a reasonable possibility or not. However, the implications of the alternative view were fully considered, and care was taken to avoid conclusions leaning too heavily on such cases.

4. CONCLUSIONS AND DISCUSSIONS

A glance at Table 1 is enough to see that no abnormality of behaviour can be ascribed to any particular defect or combination of defects of the inner ear. Circling occurred in animals with severe malformations (Nos. 4, 5, 7) as well as with only moderately affected or normal inner ear (Nos. 6, 3). Further, the abnormalities of four circling mice (Nos. 1, 2, 3, 6) have their matches among the non-circling ones. The same is true of hyperactivity and jerking movements of the head. Swimming ability is impaired in all but two (Nos. 27, 30) animals with abnormal saccule or utricle or both. This is to be expected from the importance of the two maculae in maintaining normal position. But at the same time there are four mice (Nos. 14, 17, 18, 20) with similar impairment of swimming ability which have apparently normal ears. Again head tilting is one of the classical symptoms of unilateral damage to the inner ear, and Lyon (1951) has shown that both saccule and utricle play a part in its origin. It should not have occurred in animals with bilateral defects or no defects of these structures (Nos. 2, 3, 5, 6, 14, 20). Nor should the position of head be normal in animals with unilateral defects (Nos. 1, 7, 27). Moreover, mice with similar defects (Nos. 12 and 13; 15 and 16) should not hold their heads tilted in different directions. The only statement that can be made with any confidence is that the tilting condition observed here is different from that in the pallid mutant not only in the manner mentioned before (see Materials and Methods), but also in that the tilt does not invariably occur in the direction opposite to that of the more affected ear. If anything, the relationship between the side affected and the side tilted comes closer to what would be expected on the basis of extirpation experiments, that is the side with worse damage is more frequently lowered.

It is equally clear from Table 1 that the abnormalities of the inner ear are not entirely unrelated to those of the behaviour. On the whole they are severe in animals with severely affected behaviour (Nos. 1–7), moderate in animals with moderately affected behaviour (Nos. 8–22), and light or absent in animals with normal behaviour (Nos. 23–30). This is a strong indication that they have a common origin, or that at least their causes are closely related.

If none of the abnormalities of behaviour can be attributed to defects of the inner ear what, then, is their anatomical basis? The likelihood that they originate in the central nervous system is very strong. Such a possibility has been suggested before in connexion with mutants of the degenerative type (Grüneberg, 1952; Deol, 1954), but owing to the lack of any corroborative evidence, direct or indirect, it has not received much attention. No anatomical investigation of the brain in kinky mice was attempted, and none is being contemplated: it is notoriously difficult to demonstrate any but the grossest malformations even in such a relatively simple brain as that of a mouse. But strong circumstantial evidence converges from three directions to indicate that the brain of kinky mice is in all probability affected. First, it is a well-established fact in experimental embryology that the differentiation of the otic vesicle into a normal inner ear depends on the inductive influence of the neural tube in the region of the myelencephalon. Studies on the mutants kreisler, dreher, splotch and loop-tail have shown that the mouse is no exception to this (Deol, 1964*a*, *b*, 1966): it was possible to deduce the existence of the abnormalities of the neural tube from those of the inner ear and vice versa. The nature of the malformations in kinky mice points to an implication of the neural tube. The otic duct is very poorly developed, and the otic sac in most cases is confined to the otic capsule, indicating grossly retarded development at a stage when the influence of the neural tube is paramount. Moreover, the malformations of the inner ear are extremely variable, and frequently asymmetrical. This suggests that they are remote effects of the gene, for the primary effects would tend to be more constant and symmetrical. An inductive relationship between the neural tube and the inner ear would also explain the close association between abnormal behaviour and malformations of the inner ear referred to earlier on. Secondly, fused (*Fu*), an allele of kinky, is known to act on the nervous system (Theiler & Gluecksohn-Waelsch, 1956), and it is unlikely that the manner of action of the two genes is different. Thirdly, a satisfactory explanation of the origin of the abnormalities of the inner ear must take into consideration the malformations of the axial skeleton, which are a constant feature of kinky mice. The assumption of a primary defect of the neural tube, not confined to the myelencephalon but covering its entire length, meets this requirement well, for it has been shown that the spinal cord plays an important part in the induction of cartilage in its vicinity (Lash, Holtzer & Holtzer, 1957; Avery, Chow & Holtzer, 1956).

The question arises whether the abnormal behaviour of other circling mutants may not also indicate a primary involvement of the neural tube. The last two of the above three lines of evidence are peculiar to the kinky mutant, for the other genes do not have known mutant alleles, and are only rarely pleiotropic in action. But the first argument can be applied to all mutants of the morphogenetic type. The faulty differentiation of the inner ear in these mutants points to an abnormality of the neural tube. Whether this presumptive abnormality would be identifiable by such simple means as were employed in the studies of the kreisler and dreher mutants (Deol, 1964*a*, *b*) is another matter. It would certainly not be the same in all mutants, for it cannot always be a question of the absence of the inductive influence of the

neural tube, as appears to be the case with the kreisler mutant, in which the morphogenesis of the inner ear takes place along random lines. The malformations of some mutants, such as fidget (Truslove, 1956), follow a distinct pattern, signifying a modification of the inductive stimulus rather than its absence. The degenerative mutants pose a more difficult problem, for there are no embryological or genetical pointers here. However, their overall resemblance to the morphogenetic mutants is so great that it is suggested that the anatomical basis of their behaviour is also some abnormality of the central nervous system. But this abnormality would have to be such that either it does not affect the inductive properties of the neural tube or it makes its appearance after the morphogenesis of the inner ear has taken place. There would be still be the degeneration of the neural epithelium, stria vascularis and ganglion cells in the inner ear to explain. Studies on the mutants kreisler (Deol, 1964*a*), splotch (Auerbach, 1954) and dancer (Deol & Lane, unpublished) indicate that the abnormalities of the neural tube may be accompanied by those of the ganglia in the affected region. It is possible that the spiral and vestibular ganglia in the degenerative type mutants are similarly involved, but their abnormality is such that not only is the life or the period of active function of the ganglion cells themselves shortened, but the same happens to all other structures that are dependent on the ganglion cells for their development. This would account for the degeneration of the neural epithelium, which forms in response to contact with the ganglion cells or their external processes, as well as the stria vascularis, for even a casual study of the differentiation of the organ of Corti and stria vascularis leaves little doubt that their development is governed by the same factors. In fact the two may be regarded as homologous structures.

Although the putative abnormality of the central nervous system remains unidentified, something can be said about its probable location. Evidence from the kreisler and dreher mutants (Deol, 1964*a*, *b*) indicates that the myelencephalon and cerebellum are the most likely regions. The critical part may be affected by itself, or it may be included in a widespread abnormality of the brain or the whole of the central nervous system. This probably accounts for its being subject to the action of such a large number of genes.

The question arises whether the waltzing syndrome may not be centred on hyperactivity. It is possible that the circling trait is the product of inherited hyperactivity and growing up in the confined space of a mouse cage. It is also possible that circling mice drown because they are in a continuous state of agitation. The specific gravity of a mouse is below one, and dead mice always float. The defects of the saccule and utricle would only impair the swimming ability, not eradicate it, as is borne out by a number of animals in Table 1.

SUMMARY

A close examination of reports on circling mutants of the mouse suggested that the commonly held view that the abnormalities of the inner ear are responsible for the waltzing syndrome was probably without foundation. It was thought that a study

of kinky ($Fu^{ki}/+$) mice might resolve the doubt, because this mutant has the widest range of abnormalities of the inner ear and behaviour. The results showed that correlation between the two types of abnormalities, although high, was far from complete. This is interpreted as signifying that they are related collaterally rather than lineally. It is argued that they both originate in some primary abnormality of the central nervous system, and circumstantial evidence is presented in support of this argument. It is further suggested that the mode of gene action is essentially similar in all circling mutants, that is the abnormalities of the inner ear are consequent on some early abnormality of the neural tube and the ganglia in the region of the otic vesicle.

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REFERENCES

Avery, G., Chow, M. & Holtzer, H. (1956). An experimental analysis of the development of the spinal column. V. Reactivity of chick somites. *J. exp. Zool.* **132**, 409–426.

Bast, T. H. & Anson, B. J. (1949). *The Temporal Bone and the Ear*. Springfield: Charles C. Thomas.

Caspari, E. & David, P. R. (1940). The inheritance of a tail abnormality in the house mouse. *J. Hered.* **31**, 427–431.

Deol, M. S. (1954). The anomalies of the labyrinth of the mutants varitint-waddler, shaker-2 and jerker in the mouse. *J. Genet.* **52**, 562–588.

Deol, M. S. (1956). The anatomy and development of the mutants pirouette, shaker-1 and waltzer in the mouse. *Proc. R. Soc.* B, **145**, 206–213.

Deol, M. S. (1963). The development of the inner ear in mice homozygous for shaker-with-syndactylism. *J. Embryol. exp. Morph.* **11**, 493–512.

Deol, M. S. (1964*a*). The abnormalities of the inner ear in kreisler mice. *J. Embryol. exp. Morph.* **12**, 475–490.

Deol, M. S. (1964*b*). The origin of the abnormalities of the inner ear in dreher mice. *J. Embryol. exp. Morph.* **12**, 727–733.

Deol, M. S. (1966). The influence of the neural tube on the differentiation of the inner ear in the mammalian embryo. *Nature, Lond.* **209**, 219–220.

Deol, M. S. & Robins, M. W. (1962). The spinner mouse. *J. Hered.* **53**, 133–136.

Dunn, L. C. & Caspari, E. (1945). A case of neighbouring loci with similar effects. *Genetics*, **30**, 543–568.

Goldin, A. (1947). On the neurological effect of some chlorinated tertiary amines. *Fedn Proc. Fedn Am. Socs. exp. Biol.* **6**, 333.

Goldin, A., Noe, H. A., Landing, B. H., Shapiro, D. M. & Goldberg, B. (1948). A neurological syndrome induced by administration of some chlorinated tertiary amines. *J. Pharmac. exp. Ther.* **94**, 249–261.

Grüneberg, H. (1943). Two new mutant genes in the mouse house. *J. Genet.* **45**, 22–28.

Grüneberg, H. (1952). *The Genetics of the Mouse*, 2nd ed. The Hague: Martinus Nijhoff.

Grüneberg, H., Hallpike, C. W. & Ledoux, A. (1940). Observations on the structure, development and electrical reactions of the internal ear of the shaker-1 mouse. *Proc. R. Soc.* B, **129**, 154–173.

Hertwig, P. (1942). Sechs neue Mutationen bei der Hausmaus in ihrer Bedeutung Für allgemeine Vererbungsfragen. *Z. KonstitLehre*, **26**, 1–21.

Hertwig, P. (1944). Die Genese der Hirn-und Gehörorganmissbildungen bei rontgenmutierten Kreisler-Maüsen. *Z. KonstitLehre*, **28**, 327–354.

Lash, J., Holtzer, S. & Holtzer, H. (1957). An experimental analysis of the development of the spinal column. VI. Aspects of cartilage induction. *Expl. Cell Res.* **13**, 292–303.

Löwenstein, O. (1936). The equilibrium function of the vertebrate labyrinth. *Biol. Rev.* **11**, 113–145.

LYON, M. F. (1951). Hereditary absence of otoliths in the house mouse. *J. Physiol., Lond.* **114**, 410–418.

LYON, M. F. (1953). Absence of otoliths in the mouse: an effect of the pallid mutant. *J. Genet.* **51**, 638–650.

LYON, M. F. (1958). Twirler: a mutant affecting the inner ear of the house mouse. *J. Embryol. exp. Morph.* **6**, 105–116.

LYON, M. F. (1960). Zigzag: a genetic defect of the horizontal canals in the mouse. *Genet. Res.* **1**, 189–195.

PROSSER, C. L. (1950). *Comparative Animal Physiology* (Ed.). Philadelphia: W. B. Saunders Co.

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

STEIN, K. F. & HUBER, S. A. (1960). Morphology and behavior of waltzer-type mice. *J. Morph.* **106**, 197–203.

THEILER, K. & GLUECKSOHN-WAELSCH, S. (1956). The morphological effects and the development of the fused mutation in the mouse. *Anat. Rec.* **125**, 83–104.

TRUSLOVE, G. M. (1956). The anatomy and development of the fidget mouse. *J. Genet.* **54**, 64–86.