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Snell's waltzer, a new mutation affecting behaviour and the inner ear in the mouse

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1. INTRODUCTION

In July 1959 two mice with abnormal behaviour were found in a litter of six in the congenic subline B10.HA(33NX) of the C57BL/10ScSn inbred strain maintained by George D. Snell at The Jackson Laboratory, Bar Harbor. The litter and the parents were turned over to one of us (MCG) for genetical tests. It was found that the abnormal behaviour was caused by a new recessive mutation in linkage group II close to the gene short ear (*se*). The new gene was named Snell's waltzer, and assigned the symbol *sv*. Anatomical investigation revealed that the inner ear in mutant mice was also abnormal. This paper gives the results of genetical and anatomical studies as well as an account of the behaviour of *sv*/*sv* animals.

2. GENETICS

The original pair produced five more litters containing a total of 29 mice, of which six had abnormal behaviour. One of these, a female, was outcrossed to an *se*/*se* male of the SEC/1-*se* inbred strain, and produced 44 F_1 offspring, all of which were normal. Some of the F_1 animals were intercrossed and the results showed that *sv* is a recessive gene with simple mendelian inheritance, and is closely linked with *se* (Table 1, Cross 1).

To obtain *sv* and *se* on the same chromosome and to get a first estimate of the amount of recombination between them, *se*+/*se*− offspring of the intercross were mated with +*sv*/−*sv* offspring and five or more progeny were classified. One out of 24 such matings produced some *se*/*se* offspring, indicating that one parent was +*sv*/*se sv*, and the other 23 matings produced only wild type. From these data the recombination fraction p, and its variance can be estimated by means of the formulas

$$p = \frac{a}{2N-a}$$

and

$$Vp = \frac{p(1-p)(1+p)^2}{2N},$$

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Table 1. *Results of crosses showing the inheritance of* sv *and linkage between* sv *and* se.

Cross	Parents ♀	Parents ♂	Offspring +	Offspring *se* +	Offspring + *sv*	Offspring *se sv*	Total	Recombination %	Standard error	Segregation of *sv* +/−	Segregation of *sv* *sv*/*sv*	χ^2
1	$\frac{se\ +}{+\ sv}$	$\frac{se\ +}{+\ sv}$	259	139	108	0	506			398	108	3·607
2	$\frac{se\ +}{+\ sv}$	$\frac{se\ sv}{se\ sv}$	3	76	65	1	145	2·76	1·36	79	66	1·165
3	$\frac{se\ +}{+\ sv}$	$\frac{se\ sv}{se\ sv}$	3	113	87	3	206	2·91	1·17	116	90	3·282
4	$\frac{se\ sv}{+\ +}$	$\frac{se\ sv}{se\ sv}$	158	1	5	85	249	2·41	0·97	159	90	13·510
5	$\frac{se\ sv}{se\ sv}$	$\frac{se\ +}{+\ sv}$	0	58	42	0	100	0·00		58	42	2·560
6	$\frac{se\ sv}{se\ sv}$	$\frac{se\ sv}{+\ +}$	46	0	1	10	57	1·75	1·74	46	11	21·491

Recombination in ♀♀ (crosses 2, 3, 4) = 16/600 = 2.67 ± 0.66.
Recombination in ♂♂ (crosses 5, 6) = 1/157 = 0.64 ± 0.63.

where a is the number of mice found to be carrying a recombinant chromosome and N is the number of mice tested. The data give an estimate of

$$p = 1/(96-1) = 1{\cdot}05 \pm 1{\cdot}05\%.$$

Mice homozygous for *se* and *sv* were recovered from descendents of the *+sv/se sv* mouse and were used to produce backcrosses in coupling and repulsion (crosses 2, 3, 4, 5, 6, Table 1). These crosses gave combined estimates of recombination of $2{\cdot}67 \pm 0{\cdot}66\%$ for females and $0{\cdot}64 \pm 0{\cdot}63\%$ for males. The difference between the sexes is not significant by the χ^2 test ($\chi^2 = 2{\cdot}34$, $P > 0{\cdot}05$), but it is consistent in all crosses, and the lack of significance may be due to the small number of offspring measuring recombination in males.

There is a deficiency of *sv/sv* offspring in all crosses, almost certainly due to loss of this class prior to classification. In the repulsion crosses (1, 2, 3, and 5) the deviation from expectation is not significant but in the coupling crosses (4, and 6) there is a very large deficiency of *sv/sv* animals. Since there is a deficiency of *se/se* animals as well, this is probably the result of the combined deleterious effects of the two genes in the *se sv/se sv* class.

In the matings that produced the data of Table 1 some animals also carried the mutant gene luxoid (*lu*), known to be located about 16 units from *se* (Green, 1961). These matings give information on the linear order of *lu*, *se*, and *sv* (Table 2). Luxoid is recognizable in heterozygotes by an abnormal first toe on one or both hind feet, but penetrance is incomplete. The normal-toed recombinants between *se* and *sv* were progeny tested for *lu* (except for one + + + mouse in line 2, Table 2). By this means the rarest pair of complementary classes in each cross was identified, and assuming these to be the double crossover classes, we conclude that the order most compatible with the data is *lu*, *se*, *sv*.

Another similar mutation discovered by Miss Janice L. Southard in the C3H/HeJ strain in 1958 at The Jackson Laboratory, was found to be an allele of *sv*. Crosses between homozygotes for the two mutations gave 25 offspring, all abnormal. The C3H mutant appeared to be very similar to or identical with the one described here and has been discarded. Two sets of matings with it of the type *sv/+ × sv/sv* gave *sv/+* and *sv/sv* offspring in the proportion 232:162. The deficiency of *sv/sv* is roughly comparable to that shown in Table 1.

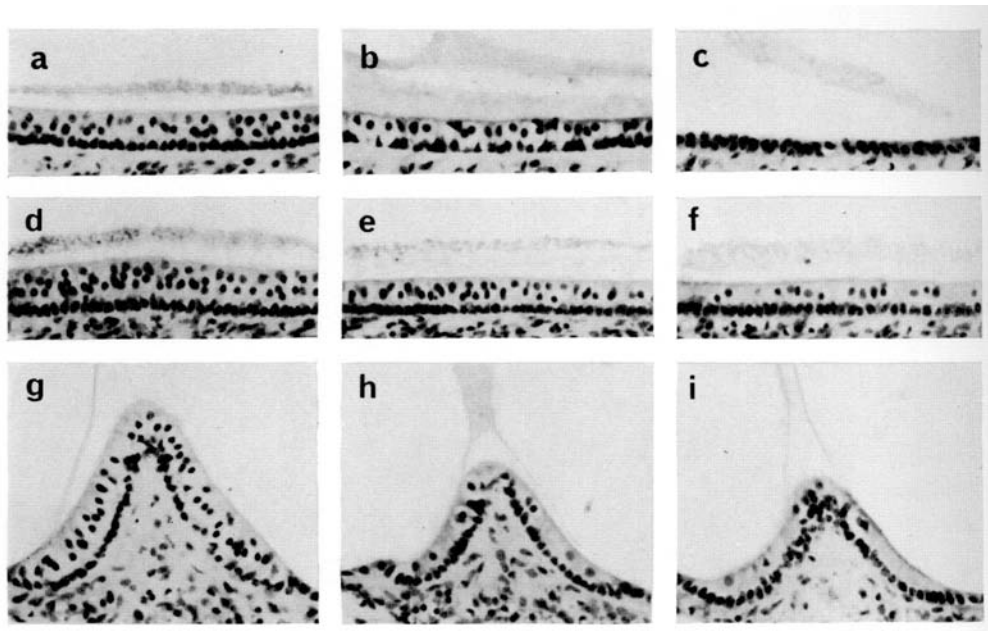
3. EFFECTS ON BEHAVIOUR

The behaviour of *sv/sv* mice closely resembles that of other mutants of the shaker-waltzer group. It is characterized by deafness, hyperactivity, jerking movement of the head, a strong tendency to run in circles and an inability to swim. Deafness appears to be present from the beginning and is total. The animals fail to respond by means of pinna reflex to any sound at any time of their life. Hyperactivity is noticeable throughout the waking period, except for brief intervals mainly during feeding and drinking. Jerking movements of the head occur mostly in the vertical plane. The head is often held still for an instant at its highest position. As a rule

Table 2. *Results of matings to determine the order of* lu, se *and* sv. *Offspring are classified for* lu *by phenotype except as indicated in footnotes. For cross numbers see Table 1*

Cross	Parents ♀	Parents ♂	Offspring *lu* + +	+ *se sv*	*lu se sv*	+ + +	*lu* + *sv*	+ *se* +	*lu se* +	+ + *sv*	Total
6, part of 4	$\frac{lu\ +\ +}{+\ se\ sv}$	$\frac{+\ se\ sv}{+\ se\ sv}$	46	77	4	123	5*	1**	0	0	256
3, part of 5	$\frac{lu\ +\ sv}{+\ se\ +}$	$\frac{+\ se\ sv}{+\ se\ sv}$	2	3**	0	1	24	154	11	99	294

* Includes 3 shown to be *lu*/+ by progeny test.
** Shown to be lu^{+} by progeny test.



Transverse sections of the saccular macula (*a*, *b*, *c*), utricular macula (*d*, *e*, *f*) and the posterior crista (*g*, *h*, *i*) of a 216-days-old normal mouse (*a*, *d*, *g*), its *sv*/*sv* littermate (*b*, *e*, *h*) and a 582-days-old *sv*/*sv* mouse (*c*, *f*, *i*). Magnification × 300.

no preference is shown for a particular direction during circling. The same animal may describe clockwise circles at one time and counter-clockwise at another. The animals are unable to remain on the surface of the water even when they are gently placed on it. They at once go under, gyrate about in all directions, and would undoubtedly drown if not quickly rescued. Classification is possible from the age of one week onwards.

4. EFFECTS ON THE INNER EAR

The following account is based on serial sections of the inner ear of nine *sv/sv* and five normal mice ranging in age from 12 to 582 days. All but one normal animal were litter-mates of the mutant mice used. Since no abnormalities were observed in mutant mice at the age of 12 days it was not considered necessary to examine earlier stages. The histological technique employed has been described in detail previously (Deol, 1954). The two ears were sectioned separately, the left one in a plane parallel to the modiolus in the cochlea and the right one transversely.

The abnormalities of the inner ear in *sv/sv* mice consist in degeneration of certain parts, the gross differentiation of the organ being normal. They first appear in the organ of Corti in the cochlea. This structure seems to develop normally up to the age of 12 days but the hair cells begin to degenerate soon afterwards, so that by 18 days the degeneration has made considerable progress and a large number of the hair cells have disappeared. Dedifferentiation of the supporting elements follows the loss of hair cells. In old age even the dedifferentiated cells degenerate in some parts of the cochlea, and the basilar membrane lies practically bare. The tectorial membrane loses touch with the organ of Corti when the hair cells degenerate, becomes distorted at its free end and starts floating in the otic fluid. The degeneration of the stria vascularis seems to run parallel to that of the organ of Corti. The stria gradually loses its vascularity and becomes thinner. In old animals it is often only a single cell thick in places. The degeneration of the spiral ganglion becomes noticeable soon after the loss of hair cells. The density of cells in the ganglion gradually decreases until, at the age of about one year, the ganglionic cavity in the modiolus is virtually empty.

In the saccule the macula appears to be fairly normal up to the age of about three weeks. Degeneration of the hair cells sets in soon afterwards, and proceeds gradually. Some of the cells disappear and the others enlarge to become rounded (Plate I, Figs. a and b). The hairs are usually lost in the early stages. The otolith membrane becomes disorganised and the otoliths may be displaced. In old animals most of the hair cells have disappeared, leaving the layer of supporting cells uncovered (Plate I, Fig. c).

The degeneration in the macula of the utricle does not begin until the age of about three months and then proceeds much more slowly than in the macula of the saccule. The loss of hair cells in old age is also not so great (Plate I, Figs. d, e, and f), although none of the surviving cells can be described as normal. Nor is the disorganization

of the otolith membrane and the displacement of the otoliths so common. In other respects the degeneration of the utricular macula is similar to that of the saccular macula.

All three cristae are affected to about the same extent. The degeneration of the neuro-epithelium takes place in much the same way as in the two maculae. It sets in at about the same time as in the saccular macula, that is, much earlier than in the utricular macula. It progresses rapidly during the first two months or so, but then slows down, so that the difference between the cristae of six months old and one year old mice (Plate I, Figs. g, h, and i) is comparatively small.

The vestibular ganglion is also affected, but not in the same way as the spiral ganglion, nor quite so early in life. The majority of its cells suffer considerable reduction in size, although a few seem to remain of normal size to the end. The reduction is progressive, and proceeds slowly. There is no actual thinning out of the ganglionic body, in contrast with the spiral ganglion.

5. DISCUSSION

The abnormalities of behaviour observed in Snell's waltzer mice are usually referred to as the shaker-waltzer syndrome. Mutations causing this syndrome, either complete or partial, are exceptionally frequent in the mouse. Nearly thirty such genes have already been discovered (Sidman, Green & Appel, 1965). Their effects always include abnormalities of the inner ear. These abnormalities may be degenerative, with breakdown of certain structures following normal differentiation, or morphogenetic, with differentiation of the whole ear or a part of it taking place along faulty lines. On this basis the shaker-waltzer group of mutants has been divided into degenerative and morphogenetic classes (Grüneberg, 1956). Snell's waltzer clearly falls into the degenerative class.

Within the degenerative class there are some mutants with such distinctive patterns of degeneration that they can be identified by them. For instance, jerker (*je*) is the only mutant in which the organ of Corti, the saccular macula and the utricular macula degenerate while the cristae remain normal, and varitint-waddler (*Va*) is the only mutant in which the organ of Corti, the saccular macula and the cristae degenerate while the utricular macula remains normal (Deol, 1956). In other mutants the pattern of degeneration may be so similar as to make identification by pathological features virtually impossible. This is the case with waltzer (*v*) and shaker-2 (*sh-2*), in both of which the organ of Corti and the saccular macula degenerate while the utricular macula and the cristae remain normal (Deol, 1956). Snell's waltzer is a mutant of the former type, having a degenerative pattern not found elsewhere. It is the only mutant in which the entire neuro-epithelium in the inner ear degenerates.

It has been suggested by Deol (1966) that the abnormal behaviour and defects of the inner ear in shaker-waltzer type of mutants are probably independent effects of the gene, and not related in a cause and effect manner. No observations made during the present study conflict with this view.

SUMMARY

A new recessive gene affecting behaviour and the inner ear in the mouse has been discovered. It was named Snell's waltzer, and assigned the symbol *sv*. It is in linkage group II, about two map units from short ear (*se*) on the side opposite to that of luxoid (*lu*). The behaviour of *sv*/*sv* mice closely resembles that of other members of the shaker-waltzer group. The abnormalities of the inner ear consist in degeneration of certain parts following normal morphogenesis. The entire neuro-epithelium —that is, the organ of Corti, the two maculae and the three cristae—is affected, and this feature distinguishes it from other degenerative type mutants of this group.

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