

VACILLANS, A NEUROLOGICAL MUTANT IN THE HOUSE MOUSE LINKED WITH BROWN

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(With One Text-figure)

(Received 6 March 1955)

The mutant to be described in this paper was first observed during December 1952 in a litter of seven, containing four mutants, in the eighteenth generation of the DBA/1 inbred strain at the Institute of Biology, University of Chile. Presumably it arose by spontaneous mutation some generations before its segregation was apparent.

The mutant seems to differ in one trait or another from all mutants previously described, and therefore it is probably a new one. The name 'vacillans' is adopted because it describes the condition. The mutant behaves genetically as a recessive and the symbol *vc* is proposed. The description of vacillans will prove it a good example of 'locomotor disturbances (forming) part of very diverse and highly intriguing syndromes', to use Grüneberg's expression.

DESCRIPTION OF VACILLANS

The following description is based on mutants coming from the isogenic DBA/1-*vc* strain. But cross-bred mutants obtained from type of mating 5 (Table 2) differ from them only in a few traits to be mentioned; the general behaviour has not changed with the cross. The syndrome involves the muscular co-ordination, behaviour and reflexes as well as some metabolic abnormalities.

By the ninth day, when the young begin to be active, vacillans sometimes fall sideways and roll over. Walking may start a day after the normal sibs. Vacillans can be recognized with certainty at the age of 14 days; when placed on the observation table they show an initial reluctance to move, and when they start moving a violent tremor is evident. While walking the hindquarters swing sideways and the mouse may roll over. With increasing age the instability lessens and a 'duck-gait' sets in gradually; the toes are spread out, and the tail is held vertical. Vacillans young are often undersized, and then they also are markedly asthenic and excitable.

By the time of weaning vacillans have acquired the adult gait. The inco-ordination is mainly confined to the posterior regions and, when walking, the tail is kept horizontal and stiff. Occasionally, but specially when the body is stretched, they show intention tremor, which is coarse, general and sometimes violent. The tremor can be made to disappear by restraining the tail of the animal.

Under certain conditions the ataxia is more manifest; on a narrow bridge they often fall and advance very little, mainly by straddling rather than by walking on the bridge.

That the sense of equilibrium exists is shown by the proper use of the tail for balancing on the bridge. The performance does not improve with practice.

Three psychic traits are noteworthy. In a site new to them vacillans remain irresolute; in this they resemble shaker-1 mice. The initial phase gives way to erratic movements which persist after return to their cages; this movement can also be elicited by mere handling of the cages. During this erratic movement the sight is fixed on the ground, the pinnae are erected, and on coming near the edge of a table vacillans are apt to fall off it.



Fig. 1. Typical attitude of vacillans when suspended by the tail. The mouse is not showing the placing reaction.

Vacillans are also docile mice, the aggressive instinct being diminished. Males will never attack and, if attacked, will at most reluctantly respond. Anoestrous females will not fight a persistent male. Finally, the maternal instincts are not normal. The female usually does not care for the newborn either by cleaning them, eating amnions and placentae, or collecting them in a nest. All this evidence points to a mental deficiency in vacillans.

Vacillans have some atypical reflexes. When suspended by the tail, the hands are locked together or, alternatively, they may show circling movements; the hind legs lock together after a gradual approach (Fig. 1). This attitude sets in at the age of a month; it is less pronounced in dorsal-decubitus. If, when the mice are held by the tail, a finger is placed on the back, they take excessive time to climb on to it. The linear acceleration

44 *Vacillans*, a neurological mutant in the house mouse linked with brown

reaction (Lyon, 1951) fails when the hands are locked. The abduction of the hands towards an approaching object (placing reaction) is sometimes delayed, so that it is possible to touch the nose with the object before the arms are stretched, especially if the hands are locked (Fig. 1). To conclude, the normal reactions are present though slowed down, as was pointed out with the bridge test.

Some other tests further indicate that the vestibular function in *vacillans* is normal. They swim, although they rapidly become tired and sink. They show rotational dizziness, after spinning in a centrifuge. At any time, if they are placed on their back, they regain easily the upright position. A peculiar trait is that, if a board on to which they have been put is moved up and down, they suffer a general tonic-clonic contracture.

Hearing is already subnormal as from 45 to 60 days old. But in cross-bred *vacillans* hearing in one litter was still normal at the age of 3 months, and in another a mild hypoacuity appeared only at 5½ months.

Table 1. *Muscular vigour of vacillans*

Phenotype	Sex	Age (months)	Dynamometer reading (g.)	Force
				Cross-surface of muscle
<i>vc</i>	♂	2.5	100	20
+			123	17
+			140	19
<i>vc</i>	♂	3	98	14
+			166	21
+			285	35
<i>vc</i>	♀	3.5	90	13
+			330	39
<i>vc</i>	♂	3.5	103	17
+			270	33
<i>vc</i>	♂	5	115	15
+			350	37
<i>vc</i>	♀	8.5	110	15
+			124	14
<i>vc</i>	♂	9	98	12
+			140	15
<i>vc</i>	♀	12	110	15
<i>vc</i>			110	15
+			175	18

Vacillans are often underweight at birth. In large litters, where their birth weight is normal, growth from about 14 days onwards always lags behind that of normal sibs. Small litters may not show this effect, which points to competition with normal sibs rather than difficulty in suckling as the cause of the retarded growth in *vacillans*. Adult weight is roughly three-quarters of the normal. Quite apart from the smaller size two important facts are a drastic diminution of all body fat and a reduction of the muscular mass. Consequently, *vacillans* look slim and kyphotic, and keep the body much more clear of the ground than normal mice. Because this external appearance has been reproduced in adult normal mice by fasting, it is surmised that *vacillans* are affected by malnutrition.

As to muscular vigour, *vacillans* give the impression of being weak. This was tested by recording with a dynamometer the maximum force exerted by the mouse when pulled by the tail. In most tests animals of the same litter were used. The ratio of the force to the area of cross-section of the muscles (taken as proportional to the two-thirds root of the weight) was then obtained for each animal. The data are given in Table 1. They indicate

that up to the age of 5 months vacillans have only about half the muscular strength of normals. From $8\frac{1}{2}$ months of age the relative strengths of mutant and normal, when measured in this way, are approximately equal. This is probably a spurious equality due to the fact that normals put on more fat and so grow heavier without increasing the size of their muscles. The dynamometer readings given by the older animals were always lower for the mutant than for the normal. In addition, the younger and older normals gave lower readings than the middle-aged ones, whereas the vacillans all gave approximately equal readings.

Observation of muscle from vacillans and from normals in saline under the phase-contrast microscope, however, disclosed no appreciable differences.

In the isogenic strain, in which the mice have a dilute, brown, non-agouti coat (d/d ; b/b ; a/a), vacillans acquire a brownish hue on the haunches, specially the males; hair of that region, examined by the technique described by Russell (1946), shows that the pigment is of a lighter brown than normal, thus explaining the colour of vacillans mice. In cross-bred vacillans the coat colour on the haunches is altered: agouti ($A/-$) and non-agouti (a/a) animals possess a brownish tint, chocolate (a/a ; b/b) a lighter brown, and brown (b/b) mice a reddish.

In females a permanent ulcer develops in the anal lips (Fig. 1), but as bacteriological examination has failed to show any pathological agent, a trophic origin is rendered likely. No such ulcer has ever been found in cross-bred vacillans.

Sexual maturation of the male never occurred before 5.5 months in about six males observed; the testes of two vacillans aged 3.5 months gave an immature histological picture. Once mature, the sexual behaviour is but slightly impaired. Females mature at the normal age or slightly later, but pregnancies recur rather irregularly. In mothers which care for their young, milk deficiency can be inferred because, despite the presence of well-developed nipples in the mother, the young are not fed and die soon. The breeding behaviour in cross-breds is less uniform and is no better on the whole. One cross-bred male of normal size matured at the normal age.

As with many other pathological mutants, vacillans shows a peak of mortality at about weaning age. Afterwards their survival seems largely unaffected, at least under laboratory conditions.

A preliminary study of the central nervous system, but not including the labyrinth, conducted by Dr S. Donoso, Hospital del Salvador, Santiago, showed no gross anatomical abnormality.

GENETICS

The litters were classified at about the 14th day, when the syndrome of vacillans offers no possibility of confusion with the normal mouse. The data in Table 2 show that vacillans is a recessive gene. The history of the stocks containing shaker-1 and hairless-rhino, with which vacillans was outcrossed in matings of type 2 and 3, can be seen elsewhere (*Mouse News Letter*, 1953).

Intercrosses, in matings of types 4 and 5, obtained in Santiago and Edinburgh respectively, show a significant departure from a 3:1 ratio. As in matings 4 some degree of selection was practised, because some of the parents were recognized as heterozygotes by their progeny, the total is best adjusted by the correction of Lenz (Li, 1948). This segregation then becomes 205:38. For matings 4 (corrected) and 5 the χ^2 's are 11.36 and 8.61

46 *Vacillans*, a neurological mutant in the house mouse linked with brown

and the probabilities <0.1 and $<1\%$, respectively. The uniformity in the expressivity of the phenotype and the lack of evidence of normal overlapping suggest that the deficiency of vacillans may depend on an impaired viability rather than on incomplete penetrance. This is supported by the constitutional weakness of the mutant. In addition, DBA females are known (Grüneberg, 1952, p. 12) to provide a poor uterine environment.

The viability of vacillans is, as expected, higher in matings of type 5, in which both mothers and young benefit from heterotic vigour, than in matings 4, which take place entirely within the inbred strain; the viabilities are 0.76 and 0.56, respectively (matings 4 corrected). However, if both types of mating are compared in a 2×2 contingency table, they do not differ significantly.

Matings 5 are composed of ten families, which are homogeneous between them, and disclose no apparent effect of parity or age of the mother on the ratio of normal to vacillans.

The backcrosses, in matings of types 6 and 7, accord well with expectation, though the numbers are small. For matings 8 offspring were fostered from birth. The sex ratio in the total of vacillans is very near unity.

Table 2. *Segregations of vacillans*

Type of mating	Genotype of parents ♀ × ♂	Stock ♀ × ♂	Progeny							
			Normal				Vacillans			
			♀	?	♂	Total	♀	?	♂	Total
1	+ / + × vc/vc	DBA/1-vc	—	—	—	40	—	—	—	—
2	+ / + × vc/vc	sh-1 × DBA/1-vc	—	—	—	52	—	—	—	—
3	+ / + × vc/vc	h ² × DBA/1-vc	—	—	—	43	—	—	—	—
4	+ /vc × + /vc	DBA/1-vc	93	8	98	199	15	1	22	38
5A	+ /vc × + /vc	3 × 2	255	—	244	547	68	—	57	138
5B	+ /vc × + /vc	7 × 7	20	—	28	—	8	—	5	—
6	vc/vc × + /vc + /vc × vc/vc	DBA/1-vc	4	—	6	10	5	—	4	9
7	+ /vc × vc/vc	3 × DBA/1-vc	8	—	13	21	8	—	11	19
8	vc/vc × vc/vc	DBA/1-vc	—	—	—	—	4	—	1	5
Total			380	8	389	—	108	1	100	—

Note. ? means unsexed animals. The numbers in the stocks refer to the progenies from the types of mating under the same number.

Table 3. *Segregations of vacillans with brown*

Type of mating	Genotype of parents ♀ × ♂	Progeny				Total
		+ +	b +	+ vc	b vc	
7	+ + /bvc × bvc/bvc	21	0	1	18	40
5A	+ + /bvc × b + /bvc	234	141	6	95	476
5B	+ + /bvc × + + /bvc	47	1	2	11	61

Types of mating as in Table 2.

LINKAGE

Table 3 is the detailed account of the data in Table 2, useful for estimating the recombination between vacillans and brown. In Table 2, matings 5A include 148 albino segregants which are excluded from the classification for brown in Table 3. For estimating the recombination fraction Fisher's scoring system, as described by Carter & Falconer (1951), has been used. In matings 5 the amounts of information were adjusted to the observed viability of vacillans.

In Table 4 the conclusion of the analysis for female recombination is given. The small χ^2 indicates no deviation from the assumed recombination fraction, $p = 0.54$, as obtained

with previous approximations. The final estimate is then $5.5 \pm 1.9\%$. In agreement with this, matings 5B, where both sexes segregate, give a recombination value of $5.4 \pm 3.1\%$.

A three-point test to locate vacillans in relation to misty and brown in linkage group VIII is in progress.

Table 4. *Analysis of the female recombination data in Table 3, scored for the assumed value $\eta = 0.54$*

Type of mating	Score	Amount of information
7	-22.7	783.0
5A	24.2	1951.1
Total	1.5	2734.1
$\bar{\chi}^2$		0.0008
Estimated recombination		5.5%
Standard error		1.9%

INTERACTION OF VACILLANS WITH OTHER NEUROLOGICAL GENES

It was thought desirable to cross vacillans with some other genes similarly conditioning inco-ordination and readily available. The crosses would test for interaction and, in case the linkage relations of the second gene are unknown, for allelism.

All females in matings 3 were heterozygous for *agitans* (Hoecker, Martinez, Markovic & Pizarro, 1954), and no young from those matings suggested any interaction of vacillans with *agitans*. Homozygous vacillans males were mated with females heterozygous for *ataxia* (Lyon, 1955), kindly provided by Dr M. F. Lyon. In fifty-five young obtained there was no evidence for an interaction. Females heterozygous for *reeler* (Falconer, 1951) were mated with vacillans males; fifty-six young sired by heterozygous males and fifteen young by homozygous males showed normal behaviour. Vacillans shows no interaction with *shaker-1* in the double heterozygotes obtained from matings 2. Hearing particularly was tested in the adults and was normal.

In a double intercross for vacillans and *shaker-1* the following progeny was obtained: +, 20; *vc*, 5; *sh-1*, 6; *vc-sh-1*, 2, in good agreement with a 9 : 3 : 3 : 1 ratio. The compound is underdeveloped and moves sluggishly, the chorea and circling being less marked than in shakers. In their coarse tremor and in their gait they resemble vacillans, though the position of the hind legs recalls the shakers. When suspended by the tail the young show the disordered movement of the legs typical of shaker; after a month they lock the legs in the way vacillans does.

SUMMARY

Vacillans is a gene that conditions a complex syndrome involving the muscular co-ordination, behaviour and reflexes, as well as some metabolic abnormality. The syndrome seems to a large extent independent of the residual genotype. It behaves as a recessive, and the symbol *vc* is proposed. Vacillans is linked with *brown*, in the linkage group VIII, with a recombination value of $5.5 \pm 1.9\%$ in females. Double heterozygotes of vacillans and *shaker-1*, *agitans*, *ataxia* and *reeler* show no interaction. The compound *vc/vc; sh-1/sh-1* is described.

The author wishes to thank Profs. G. Gasić and C. H. Waddington, F.R.S., for their hospitality in Santiago and in Edinburgh, Dr D. S. Falconer for advice and criticism of the manuscript, and Mrs I. Moscoso, who found the first vacillans mice, for technical

48 *Vacillans*, a neurological mutant in the house mouse linked with brown

assistance. In Santiago the work was partially financed by a grant from the Rockefeller Foundation to Prof. Gasić, and in Edinburgh the author worked under a British Council scholarship, which he gratefully acknowledges.

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Note added in proof. After this paper went to press it became apparent to the author that the syndrome of *vacillans* compares in many traits with that of vitamin E deficiency (cf. Mason, K. E. (1954) in *The Vitamins*, eds. Sebrell, W. H. and Harris, R. S., **3**, 514-62. New York: Academic Press). Two more traits have been observed since then. First, with the kind co-operation of Dr H. H. El-Shatoury, stained sections of muscle from *vacillans* and normal mice have been examined, and the muscle of *vacillans* often showed fainter striations. Secondly, various tissues of *vacillans*, such as skeletal muscle fat and intestine, show a brownish pigmentation compared with normals. Experiments are in progress testing the dependence of the *vacillans* syndrome on vitamin E.