11 Weinhouse, S., in: Molecular Interelations of Nutrition and Cancer. pp. 167–181. Eds M. S. Arnott, J. Vaan Eys and Y. M. Wang. Raven Press, New York 1982.
12 Seamon, K. B., and Daly, J. W., in: Advances in Cyclic Nucleotide and Protein Phosphorylation Research, vol. 20, p. 1. Eds P. Greengard and G. A. Robinson. Raven Press, New York 1986.
13 Tartakoff, A. M., Cell *32* (1983) 1026.
14 Green, F., Edwards, Y., Hauri, H. P., Povey, S., Ho, M. W., Pinto, M., and Swallow, D., Gene *57* (1987) 101.
15 Darmoul, D., Lacasa, M., Chantret, I., Swallow, D. M., and Trugnan, G., Ann. Hum. Genet. *54* (1990) 191.
16 Messer, M., and Dahlqvist, A., Analyt. Biochem. *14* (1966) 376.
17 Nagatsu, T., Hino, M., Fuyamada, H., Hayakawa, T., Sakakibara, S., Nakagawa, Y., and Takemoto, T., Analyt. Biochem. *74* (1976) 466.
18 Burnette, W. N., Analyt. Biochem. *112* (1981) 195.
19 Danielsen, E. M., J. biol. Chem. *264* (1989) 13726.
20 Hauri, H. P., Roth, J., Sterchi, E. E., and Lentze, M. J., Proc. natl Acad. Sci. USA *82* (1985) 4423.
21 Triadou, N., Audran, E., Dellon, A., and Schmitz, J., in: Ion Gradient-coupled Transport, p. 367. Eds F. Alvarado and C. H. Van Os. Inserm Symposium n°26 (1986).
22 Darmoul, D., Baricault, L., Sapin, C., Rousset, M., and Trugnan, G., European Congress on Cell Biology. (Abst.) Florence, Italy 1990.

0014-4754/91/11-12/1211-05$1.50 + 0.20/0

Neuronal degeneration in the striatum of the groggy rat: A new mutant with a movement disorder

I. K. Takeuchi and Y. K. Takeuchi [a]

Department of Embryology, Institute for Developmental Research, Aichi Prefectural Colony, Kasugai, Aichi 480-03, and [a] Anatomical Laboratory, Gifu College of Medical Technology, Seki 501-32 (Japan)
Received 15 January 1991; accepted 21 March 1991

Abstract. A new mutation displaying abnormal movement was obtained in the progeny of a female Wistar rat which had been given 10 mg/kg methylnitrosourea at an early stage of the gestational period. Genetic studies revealed that the character is inherited by an autosomal single recessive gene, and we designated this mutation *groggy* (gene symbol *gr*). The abnormal movement of the groggy rat was first apparent around postnatal day 15, while the histological studies revealed the appearance of numerous necrotic neurons in the striatum of the groggy rat on postnatal days 60 and 120.
Key words. Neurological mutant; movement disorder; striatum; neuronal degeneration; Wistar rat.

Numerous mutations with neurological abnormalities have been reported in mice. In rats, however, very few neurological mutants have yet been described. In the course of studying the effects of methylnitrosourea administration to pregnant rats in terms of the development of the progeny, a new mutation was obtained displaying movement disorder. In the present study, the clinical and genetical features of this mutant rat are first described; thereafter, results of histological examination of the brain are reported.

Materials and methods

Origin of animals. A female Slc:Wistar rat was given 10 mg/kg methylnitrosourea intraperitoneally on gestational day 4 (the onset of pregnancy was designated gestational day 0). This mother bore 5 normal-appearing offspring (2 males and 3 females). These animals were randomly mated and one female gave birth to 7 offspring among which 3 animals (2 males and 1 female) displayed the same pattern of movement disorder. Then, one of the abnormal males was paired with the abnormal female, and all the offspring obtained from this pair displayed the same pattern of movement disorder. Further breeding is performed in 2 ways. Pedigreed stock is maintained by brother-sister-mating. The mutants used for the experiments are produced by mating homozygous males with heterozygous females, since the offspring of the homozygous females mated with homozygous males become dirty owing to the creeping movement of the mother during forstering.

Histological study. On various postnatal days males of the mutant rats were deeply anesthetized by ethyl ether, and killed by a cardiac perfusion with 10% formalin after the blood vessels had been flushed out with physiological saline. After perfusion, the brains were quickly removed, bisected coronally, and fixed with Bodian II solution for 24 h. They were then dehydrated with an ethanol series and embedded in paraffin wax. For the control, normal rats of the Slc:Wistar strain were sacrificed and their brains were embedded in paraffin wax by the same methods. Coronal sections 8–10 μm thick were stained with cresyl violet before light microscopic examination.

Count of necrotic neurons in the striatum. The number of necrotic neurons appearing in the striatum of the Slc:Wistar and the mutant rats during postnatal growth was counted in the coronal section of the caudate nucleus cut at the plane approximately corresponding to FIG. A29 of Craigie's Neuroanatomy of the Rat [1]. Five males were examined on each selected postnatal day, and statis-

tical analysis was performed using the Mann-Whitney U test.

Results

Clinical features and genetic observations. The abnormal movement of the mutant rat first becomes apparent around postnatal day 15. Initial clinical signs are an ambulation with dragging hindlimbs (fig. 1), and a frequent twisting of hindlimbs during locomotion (fig. 2). On postnatal day 20, the animals become less active, and frequently lie on their side with the hindlimbs extending (fig. 3). Their locomotion is unstable owing to the stiff movement of hindlimbs (fig. 4), and they frequently topple over (fig. 5). Thereafter, the symptoms become stable. Weight gain of the affected rats is somewhat retarded, and the average body weight of mutant rats on postnatal day 60 (235.0 g in male) is significantly reduced as compared with the normal littermates (345.0 g in male) ($p < 0.001$, by Mann-Whitney U test).

All F_1 offspring obtained from the mating of mutant rats with normal Wistar rats displayed normal movement throughout life. In 341 F_2 animals from $F_1 \times F_2$ mating, 87 displayed abnormal movement and the remaining 254 were normal, a close approximation of the expected value of 85.25:255.75 for 1:3 segregation (table 1). Of 506 offspring from backcross mating of F_1 animals to homozygous parents, 234 animals showed the abnormal phenotype, while the remaining 272 animals were nor-

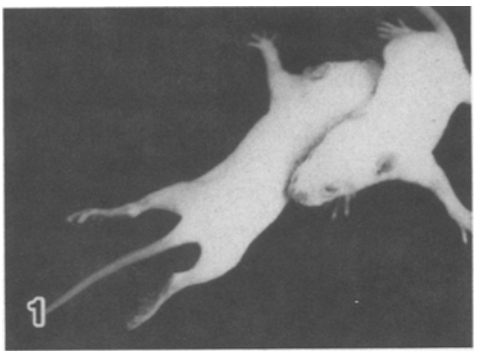

Figure 1. A groggy rat on postnatal day 15 showing ambulation with dragging hindlimbs.

Figure 2. Twisting of the hindlimbs during locomotion of the groggy rat. Postnatal day 15.

Figure 3. A groggy rat lying on his side with the hindlimbs extending. Postnatal day 20.

Figure 4. Stiff movement of hindlimbs during locomotion of the groggy rat on postnatal day 20.

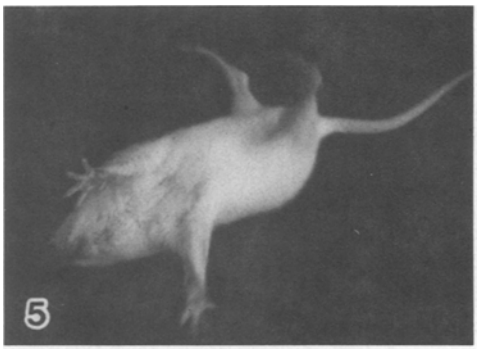

Figure 5. The groggy rat topples over during locomotion. Postnatal day 20.

Table 1. Segregation of affected animals in F_2 offspring after intercross of F_1 heterozygotes derived from crossing mutant rats and normal Wistar rats

	Affected	Unaffected	Total
Number of progeny	87	254	341
Expected value for 1:3 segregation	85.25	255.75	341

$0.95 < p < 0.90$ (χ^2 test).

Table 2. Segregation of affected animals in backcross of F_1 heterozygotes with mutant parents

	Affected	Unaffected	Total
Number of progeny	234	272	506
Expected value for 1:1 segregation	253	253	506

$0.80 < p < 0.70$ (χ^2 test).

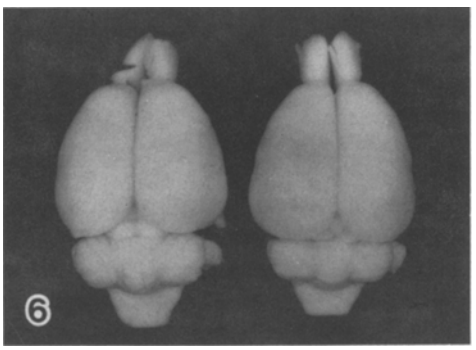

Figure 6. Brains of the groggy (*right*) and the normal Wistar (*left*) rats on postnatal day 120. The cerebellum of the groggy rat is slightly reduced in size as compared to that of the normal Wistar rat.

mal. The deviation from the expected value for 1:1 segregation of 253:253 was not significant (table 2). Thus, the character is considered to be inherited by an autosomal single recessive gene, and we designate this mutation *groggy* (gene symbol *gr*).

Table 3. Number (mean ± SD) of necrotic neurons in the caudate nuclei (C.N.) of the Slc:Wistar and the groggy rats

Postnatal day	Slc:Wistar rat Right C.N.	Slc:Wistar rat Left C.N.	Groggy rat Right C.N.	Groggy rat Left C.N.
10	–	–	0	0
15	–	–	0	0
20	0.2 ± 0.4	0.6 ± 1.3	0	0
30	1.2 ± 1.8	2.2 ± 2.9	2.6 ± 3.8	3.6 ± 2.5
40	3.0 ± 2.8	4.8 ± 4.4	1.6 ± 1.3	2.4 ± 3.6
60	0.8 ± 1.3	1.6 ± 1.7	39.2 ± 33.2[a]	56.0 ± 53.2[a]
120	1.6 ± 3.6	1.8 ± 1.6	65.6 ± 65.7[a]	68.8 ± 86.3[a]

[a] $p < 0.01$ compared with respective Slc:Wistar-rat group.

Gross morphology. Until postnatal day 40, no differences were discernible between the brains of the mutant rats and those of the normal Wistar rats. On postnatal day 60, the cerebella of some mutant rats were slightly smaller than those of the normal Wistar rats. The slight reduction in the size of the cerebellum was clearly noticed in all the brains from mutant rats on postnatal day 120 (fig. 6).

Histological observations. No noticeable histological abnormalities were found in the brains of the young mutant rats, whereas numerous necrotic neurons were observed in the striatum of the mature mutant rats. These necrotic neurons were intensely and almost homogeneously stained with cresyl violet in their cytoplasm and nuclei, and appeared to have shrunk (fig. 7). Table 3 shows the number of necrotic neurons appearing in the caudate nuclei of the Slc:Wistar and the mutant rats during postnatal growth. No necrotic neurons appeared in the cau-

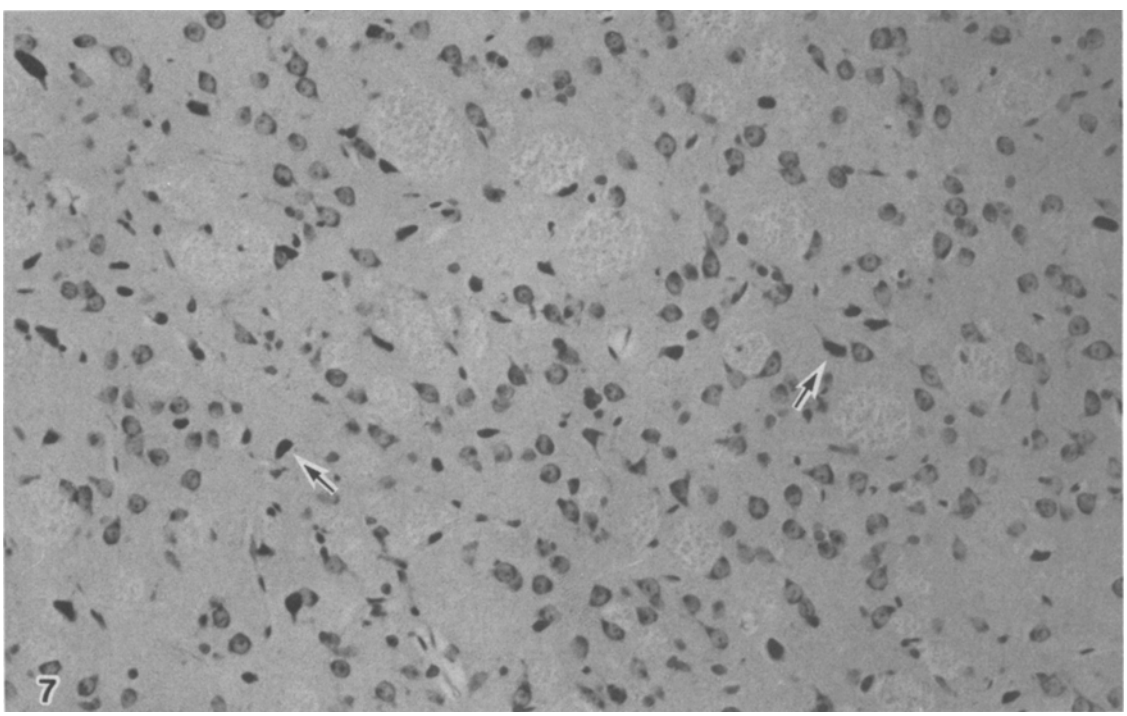

Figure 7. Coronal section of the caudate nucleus of the groggy rat on postnatal day 60. Numerous necrotic neurons, homogeneously dense and appearing to have shrunk, are found (*arrows*) (× 256).

date nuclei of the mutant rats up to postnatal day 20. On postnatal day 30 and day 40, a few necrotic neurons were found in the caudate nuclei of some of the Slc:Wistar and the mutant rats. On postnatal days 60 and 120, numerous necrotic neurons were encountered in the caudate nuclei of the mutant rats. The number of necrotic neurons varied not only among the different mutant rats but also between right and left caudate nuclei in the same mutant rat (data not shown).

Discussion

Among many kinds of mutant rodents with movement disorders, to our knowledge only one mutant mouse, *weaver*, has been reported as having abnormalities in the striatum. In the striatum of the weaver mouse, however, a massive postnatal loss of dopamine, owing to the cell death of dopaminergic neurons in the substantia nigra pars compacta, has been reported, but no neuronal degeneration was described[2-4]. Thus, the mutant rat described in this paper, *groggy*, may be a new kind of neurological mutant displaying neuronal degeneration in the striatum.

Although the first noticeable abnormal movement appeared around postnatal day 15 in the groggy rat, the histological study of the brains during postnatal growth showed that the onset of the appearance of necrotic neurons in the striatum was around postnatal day 60. This fact suggests that the neuronal degeneration in the striatum of the groggy rat may not be the primary effect of the mutant gene, but a secondary one. At present, no obvious histological abnormalities have been observed in other regions of the brain of the groggy rat except for the striatum. Further immunohistochemical studies to identify what types of neurons degenerate in the striatum of the groggy rat may provide some clues for the elucidation of the real effect of the mutant gene.

In human beings, the occurrence of neuronal degeneration in the striatum has been reported in some extrapyramidal disorders such as Huntington's chorea, striatonigral degeneration, hereditary putaminal (striatal) necrosis, and infantile bilateral striatal necrosis, but the precise mechanism of loss of striatal neurons in these disorders remains obscure[5-8]. The groggy rat may be a useful model for studying this problem.

1 Zeman, W., and Innes, J. R. M., Craigie's Neuroanatomy of the Rat. Academic Press, New York 1963.
2 Graybiel, A. M., Ohta, K., and Roffler-Tarlov, S., J. Neurosci. *10* (1990) 720.
3 Roffler-Tarlov, S., and Graybiel, A. M., in: The Basal Ganglia II, pp. 443–457. Eds M. B. Carpenter and A. Jayaraman. Plenum Press, New York 1987.
4 Smith, M. W. III, Cooper, T. R., Joh, T. H., and Smith, D. E., Brain Res. *510* (1990) 242.
5 Roos, R. A. C., in: Handbook of Clinical Neurology, vol. 49, Extrapyramidal Disorders, pp. 315–326. Eds P. J. Vinken, G. W. Bruyn and H. L. Klawans. Elsevier Science Publishers B. V., Amsterdam 1986.
6 Adams, R. D., and Salam-Adams, M., in: Handbook of Clinical Neurology, vol. 49, Extrapyramidal Disorders, pp. 205–212. Eds P. J. Vinken, G. W. Bruyn and H. L. Klawans. Elsevier Science Publishers B. V., Amsterdam 1986.
7 Druschky, K.-F., in: Handbook of Clinical Neurology, vol. 49, Extrapyramidal Disorders, pp. 493–498. Eds P. J. Vinken, G. W. Bruyn and H. L. Klawans. Elsevier Science Publishers B. V., Amsterdam 1986.
8 Jellinger, K., in: Handbook of Clinical Neurology, vol. 49, Extrapyramidal Disorders, pp. 499–518. Eds P. J. Vinken, G. W. Bruyn and H. L. Klawans. Elsevier Science Publishers B. V., Amsterdam 1986.

0014-4754/91/11-12/1215-04$1.50 + 0.20/0

Role of genetic variability in neonatal jaundice. A prospective study on full-term, blood group-compatible infants

N. Lucarini, F. Gloria-Bottini, L. Tucciarone, E. Carapella, G. Maggioni and E. Bottini

Chair of Human Development, 2nd University of Rome, School of Medicine, Via Orazio Raimondo, I-00173 Rome, Dept. of Child Health, 1st University of Rome, School of Medicine, Rome, and Dept. of Molecular, Cellular and Animal Biology, University of Camerino, School of Science, Camerino (Italy)
Received 22 March 1990; accepted 27 March 1991

Abstract. A series of genetic, developmental and environmental variables have been analyzed in a prospective sample of full-term newborn babies, compatible with their mothers in the major blood group systems, in order to attempt an evaluation of the effect of these variables on serum bilirubin level during the first few days of life.

Three genetic factors (PGM_1, ACP_1 and ADA) and three non-genetic variables (rise of bilirubin level during the first day of life, a mother with a history of previous abortion, and use of alcoholic beverages by the mother) have a significant predictive value for the separation of newborns with clinically relevant jaundice from other infants.

Key words. Neonatal jaundice; genetic polymorphism; risk of hyperbilirubinemia; adenosine deaminase; acid phosphatase; phosphoglucomutase.