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Newly Established Low Seizure Susceptible and Seizure-Prone Inbred Strains of Mongolian Gerbil

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Abstract: *Two inbred strains of the Mongolian gerbil with different phenotypes in seizure behaviour and coat color were newly established. LSAG/Nu has low seizure susceptibility and albino phenotypes, whereas SPBG/Nu has seizure-prone and black coat color phenotypes. LSAG was compared with SPBG as to seizure incidence and grade. Mean ages at seizure onset of LSAG and SPBG were 6 and 3 months, respectively. Seizure incidences in over 9 months old LSAG and SPBG gerbils were 37.3% (66/177) and 95.2% (118/124), respectively. LSAG has a significantly lower incidence ($p<0.001$) and grade ($p<0.001$) of seizures than SPBG. Only a few seizing LSAG gerbils exhibited myoclonus to tonic-clonic seizure progression. These results suggest that LSAG has some mechanisms which delay the onset of seizures and prevent them from becoming serious. Both strains of gerbils can be expected to be useful animal models for the study of human idiopathic generalized epilepsy.*

Key words: *epilepsy, gerbil, inbred strain*

Approximately 1% of the human population suffer from some form of epilepsy [4]. Many patients are refractory or uncontrollable by anti-epileptic drugs. Therefore, the development of useful animal models for elucidating the mechanism of epileptogenesis is desirable [1, 3]. The Mongolian gerbil was introduced in the 1960s as a new laboratory animal which exhibited spontaneous seizures in response to a variety of stimuli [11]. In the 1970s, Loskota *et al.* [9, 10] established non-inbred strains of seizure-sensitive and seizure-resistant gerbils by selective breeding, and confirmed that the seizure behaviour of gerbils is epileptic with electroencephalographic recordings, in which the spike and wave complexes were seen. Since then Mongolian gerbils have been widely used as an animal model for epilepsy. Up to the present some inbred strains of gerbil have been established in different colonies [5, 17, 18]. We have established two inbred strains of low seizure susceptible albino gerbils (LSAG/Nu, pink-eyed and white coat colored) (Fig. 1-a) and seizure-prone black gerbils (SPBG/Nu, partially white colored on the forelimbs and mandible) (Fig. 1-b) derived from our

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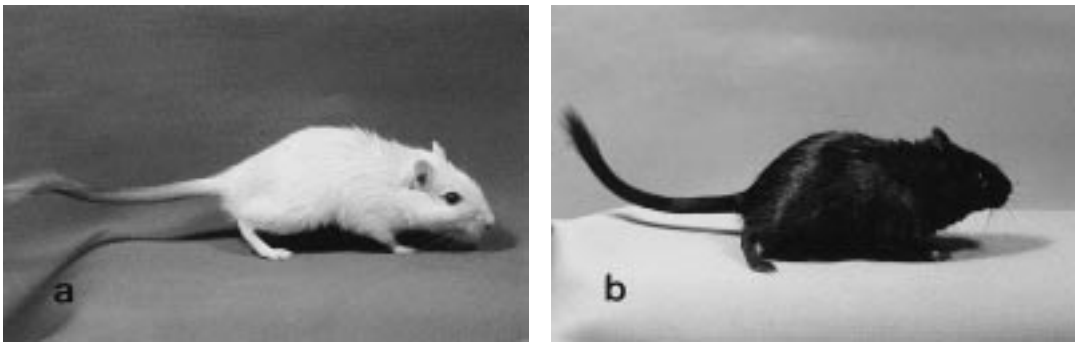

Fig. 1. Appearances of two gerbil strains, LSAG(a) and SPBG(b). They are both 4 months old males.

original breeding colony by sib-mating up to the twentieth generation. As for albino gerbils, in 1973 Robinson [14] introduced white-coated and pink-eyed albinos starting to color their tail hair at about three months of age, the so-called acromelanic albino. Since LSAG gerbils also exhibit the above features, it would seem to be the same coat color mutation as Robinson reported.

The original animals were introduced from a petshop in Yokohama, Japan to Kawasaki Medical School (Kurashiki, Japan) and sent to the Department of Physiology, Niigata University School of Medicine (Dr. Y. Sakuma). We obtained 16 gerbils (agouti: 2 males and 6 females, albino: 1 male and 5 females, black: 1 male and 1 female) from Dr. Sakuma in 1984 to make a stock colony of gerbils with coat color mutation, and started sib-mating to establish inbred strains depending on coat color. After several generations we aimed at seizure incidence and started selective breeding to establish a low seizure susceptible strain in albinos and a seizure-prone strain in black-coated gerbils. Seizure incidence of the generations F6–15, 16–20, 21–25 and 26–30 in over 9 months old LSAG gerbils were 56, 31, 44 and 32%, respectively, and those of the generations F4–10, 11–15 and 16–20 in over 6 months old SPBG gerbils were 91, 95 and 97%, respectively. In 2001, the generations of LSAG and SPBG reached F35 and F22, respectively.

The gerbils were used for the present study in accordance with the guidelines for animal experimentation of Niigata University. They were housed in plastic cages (CRJ, Tokyo, Japan, W143 × D293 × H148 mm) with bedding (CRJ, White wood flakes) and kept on a stainless steel rack and given a cubic diet, CE-2 (CLEA Japan, Inc., Tokyo, Japan) and water *ad libitum*. All feeding materials except food and drinking water were sterilized in an autoclave. Constant temperature (23 ± 2°C) and humidity (40 to 70%) were maintained. The air in the room was changed 18 times per hour and the room was illuminated with daylight fluorescent lamps for 14 h (6:00 a.m. to 8:00 p.m.) a day.

Table 1. Grades of seizure

Grade	Severity
–	No seizure
±	Twitching of vibrissae and pinnae
±	Myoclonic seizure
++	Tonic-clonic seizure

After weaning at 30 days of age the gerbils were placed in a new cage and observed for seizure occurrence for 1–2 min once or twice a week during routine care. No specific stimulation to induce seizures was applied except for the cage changing. The severity of seizures was scored in four grades as follows, –: no seizure, ±: twitching of vibrissae and pinnae, +: myoclonic seizure, and ++: tonic-clonic seizure (Table 1). The date of the first seizure observed for each grade of severity was recorded individually. The over nine months old LSAG (F14–30) and SPBG (F4–17) gerbils were compared as to the incidence and severity of seizures. Statistical analysis was performed with the χ^2-test for incidence and Wilcoxon's rank order sum test for severity.

Seizure incidences in over 9 months old LSAG and SPBG gerbils were 37.3% (66/177) and 95.2% (118/124), respectively. LSAG had a significantly lower incidence ($p<0.001$) and grade ($p<0.001$) of seizure than SPBG (Table 2). The mean age at seizure onset of LSAG was 6 months. LSAG had later seizure onset

Table 2. The incidence and grade of seizure in LSAG and SPBG

Strain	Age	n	No. of animals (%) in grades				Incidence (%)	χ^2
			–	±	+	++		
LSAG (Albino)	9M≤	177	111 (62.7)	53 (29.9)	11 (6.2)	2 (1.1)	66/177 (37.3)	100.3596 ($p<0.001$)
SPBG [a)] (Black)	9M≤	124	6 (4.8)	14 (11.3)	32 (25.8)	72 (58.1)	118/124 (95.2)	

Generation: LSAG; F14–30, SPBG; F4–17. [a)]: Significantly ($p<0.001$) severe in SPBG by Wilcoxon's rank order sum test.

Table 3. Cumulative seizure incidence in LSAG and SPBG

Age at onset (M)	LSAG (n=166) [a)]		SPBG (=96) [a)]	
	No.of animals with seizure	Cumulative incidence %	No.of animals with seizure	Cumulative incidence %
0–1	0	0	0	0
1–2	0	0	5	5.2
2–3	2	1.2	34	40.6
3–4	9	6.6	21	62.5
4–5	7	10.8	19	82.3
5–6	4	13.3	3	85.4
6–7	7	17.5	2	87.5
7–8	7	21.7	2	89.6
8–9	5	24.7	1	90.6
9–10	5	27.7	1	91.7
10≤	9	33.1	2	93.8
Total	55	33.1	90	93.8

[a)]: Gerbils observed over 9 months after birth were used, and those in which the age at onset of seizure was unclear were excluded from the data for cumulative incidence.

than SPBG (mean 3 months) (Table 3).

Many epilepsy researches using the Mongolian gerbil have compared seizure-sensitive animals to seizure-resistant ones. In almost all of these studies the gerbils were separated into two groups, seizing and non-seizing animals, by means of the seizure-inducing tests for the same colony [2, 6, 7, 12]. Only a few studies, in which established non-inbred or inbred strains produced by selective breeding depending on seizure susceptibility were used, have been reported [9, 17, 18]. The incidences of the seizure-sensitive strains reported were 97% [9], approximately 100% [17] and 100% [18]. From several reports, the mean age at seizure onset in gerbils is 77.6 days for albinos [13], and 54–70.6 days for agoutis [8, 9, 13]. The mean age at onset in LSAG was much higher than those of the above reports, and few seizing LSAG gerbils exhibited myoclonus to tonic-clonic seizure progression. Many seizing SPBG gerbils exhibited severe seizures with aging, and mean ages at the onset of myoclonus and clonic-tonic seizure were 5 and 7 months, respectively. Eighty percent of the seizing LSAG gerbils had low grade (±) seizures, whereas 88% of seizing SPBG had high grades (+ or ++). It therefore seems that LSAG has some mechanisms which delay the onset of seizures and also prevent them from becoming serious.

Loskota *et al.* [9] have reported that the mean rating (severity) of seizures was 4 (clonic-tonic seizure) at 6 months of age in the seizure-sensitive WJL/UC strain. Seizure incidence and progression in SPBG were similar to those in WJL/UC. Because details of the seizure resistant strains in the reports of Loskota *et al.* [9] and

Takeuchi *et al.* [18] are not given, the seizure characteristics of LSAG could not be compared with those of these strains. Scotti *et al.* [16] reported that the establishment of a seizure-resistant strain of gerbils is very difficult. Actually, gerbil strains which exhibit no seizures at all have not been known until now. In 1976, Robbins [13] reported that the seizure incidence was significantly lower in albino gerbils (16.1%) than in agoutis (41.5%) from the same colony. He tested the gerbils starting at 6 weeks of age and ended 10–16 weeks later. In this study, age matched LSAG gerbils (3 to 5 months old) had a lower incidence (10.8%) than the albino gerbils of Robbins's data. In the case of LSAG, the monthly number of seizure onset animals subsequently increased from 6 to 9 months old, suggesting that we should observe seizure behavior for nine months after birth to see essential seizure phenotypes in low seizure susceptible strains such as LSAG.

Concerning the seizure mechanisms in Mongolian gerbils, recent studies have reported a relationship to abnormal synaptic structure [2, 12], change in neuropeptide Y [7] in the hippocampus, a species-specific presence of the calcium-binding protein parvalbumin in the perforant path synapse [15], and differences in drug-metabolizing functions [6]. But these results, individually, do not yet clearly explain the relation of seizures to phenotype. In previous reports, almost all gerbils had seizure onset from 50 to 90 days old, which is thought to be the period of sexual maturity for this species. In view of this, we consider that Mongolian gerbils could become an animal model for human idiopathic generalized epilepsies (IGE) of adolescent onset. Juvenile myoclonic epilepsy (JME) and juvenile absence epilepsy (JAE) are both classified as subforms of IGE of adolescence. We are interested in the relationship between the gerbil seizure and JME or JAE with regard to the pathological and genetic properties.

It is not yet known whether LSAG gerbils are controlled by seizure-resistant alleles or gene(s) different from those in seizure-prone gerbils. Our data on genetic analysis suggest that at least one gene conferring low seizure susceptibility on LSAG seems to be linked with the *c* locus (Data not shown). It is thought that LSAG and SPBG will be very useful strains of gerbil for establishing an etiologic model of some type of human epilepsy and for clarifying its mechanisms.

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