Exp. Anim. 62(3), 181–187, 2013

—Original—

Identification of QTLs Involved in the Development of Amygdala Kindling in the Rat

Ryoko HASHIMOTO[1)], Birger VOIGT[1)], Yuji ISHIMARU[2)], Ryoji HOKAO[3)], Shigeru CHIBA[2)], Tadao SERIKAWA[1)], Masashi SASA[1)], and Takashi KURAMOTO[1)]

[1)]*Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Yoshidakonoe-cho, Sakyo-ku, Kyoto 606-8501, Japan*
[2)]*Department of Psychiatry and Neurology, Asahikawa Medical College, 2-1-1-1 Midorigaoka higashi, Asahikawa 078-8510, Japan*
[3)]*Institute of Animal Reproduction, 1103 Fukaya, Kasumigaura 300-0134, Japan*

Abstract: Amygdala kindling is useful for modeling human epilepsy development. It has been known that genetic factors are involved in the development of amygdala kindling. The purpose of this study was to identify the loci that are responsible for the development of amygdala kindling. To achieve this, rat strains from a LEXF/FXLE recombinant inbred (RI) strain panel were used. The phenotypes of amygdala kindling-related parameters for seven RI strains and parental LE/Stm and F344/Stm strains were determined. They included the afterdischarge threshold (ADT), the afterdischarge duration (ADD), and the kindling rate, an incidence of development of kindling. Quantitative trait loci (QTL) analysis was performed to identify linkage relationships between these phenotypes and 1,033 SNP markers. Although no significant differences in pre-kindling ADT and ADD were observed, a significant difference in the kindling rate was found for the LEXF/FXLE RI strain. Two QTLs for the amygdala kindling rate (*Agkr1* and *Agkr2*) were identified on rat chromosome 2. These findings clearly prove the existence of genetic influences that are involved in kindling development and suggest that substantial genetic components contribute to the progression of partial seizures into generalized seizures.
Key words: amygdala kindling, quantitative trait loci, recombinant inbred strains, temporal lobe epilepsy

Introduction

Kindling is produced by repeated electrical stimulation of limbic brain regions such as the amygdala or hippocampus. It has been used to study mechanisms that are associated with the progressive development of partial seizures into more complex forms such as convulsive secondary generalization [5]. Susceptibility to kindling development can be assessed by determining the kindling rate. This is defined as the number of daily stimuli that are required until the first stage 5 seizure occurs [14]. In rats, the kindling rate varies among different strains. For example, when the basolateral amygdala of the rat is stimulated by electricity once daily, a marked difference in the number of stimuli occurs. Sprague-Dawley and Brown-Norway rats are most susceptible, while Lewis rats are least susceptible [8]. Additionally, kindling-prone (FAST) and kindling-resistant (SLOW) rat strains were developed from a parent population of Long-Evans hooded and Wistar rats based on their amygdala kindling rates [15]. These observations suggested that genetic factors may be involved in inter-strain differences in kindling development.

Several biochemistry and molecular biology studies

(Received 3 December 2012 / Accepted 8 January 2013)
Address corresponding: T. Kuramoto, Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Yoshidakonoe-cho, Sakyo-ku, Kyoto 606-8501, Japan

were performed to determine which genes underlie susceptibility to kindling [4, 20]. However, the loci that are responsible for susceptibility to kindling remain unidentified. Kindling-related phenotypes such as the afterdischarge threshold (ADT), the afterdischarge duration (ADD), and the kindling rate are quantitative traits (QTs). It is believed that they are controlled by multiple genetic determinants. Thus genetic mapping panels that are suitable for identifying quantitative trait loci (QTLs) are required to identify the genes of interest.

Recombinant inbred (RI) strains are derived by inbreeding different sets of F2 progeny that have been derived from a cross between two inbred strains. Each RI strain possesses a unique combination of homozygous parental genomes [2]. Reproducible segregation patterns allow the effects of environmental influences and measurement errors to be eliminated. Therefore, RI strains are a powerful tool for performing QTL analyses [13]. The rat LEXF/FXLE RI strain set consists of 34 strains that were derived by crossing strains LE/Stm and F344/Stm [21]. Using the LEXF/FXLE mapping panel, which includes the parental strains, the QTLs that are associated with propylnitrosourea-induced T-lymphomas and weight reduction of the testis that is caused by exposure to diethylstilbestrol were identified [9, 22]. Recently, we measured 74 QTs of 34 RI and two parental strains and carried out linkage analyses using a strain distribution pattern (SDP) of 232 informative simple sequence length polymorphism markers [10, 24]. Additionally, we developed an SDP that consists of 1,033 single nucleotide polymorphism (SNP) markers. The improved SDP was used to identify 3,766 recombination events for the RI strains and enabled us to find 250 significant QTLs [16].

In this study, we induced amygdala kindling in seven LEXF/FXLE RI and two parental strains and determined their kindling parameters. We then carried out QTL mapping of the kindling parameters using the SDP consisting of 1,033 SNP markers to identify genetic components that are involved in kindling development.

Materials and Methods

Animals

Male rats that were derived from the following crosses were examined: LE/Stm (n=6), F344/Stm (n=6), LEXF2B/Stm (n=6), LEXF6B/Stm (n=6), LEXF7B/Stm (n=6), LEXF10C/Stm (n=6), LEXF11/Stm (n=6), FXLE13/Stm (n=6), and FLXE16/Stm (n=5). The strains were obtained through the National BioResource Project–Rat [19]. The seven RI strains were selected based on the distances that were observed for the phylogenic trees of 34 RI strains and two parental strains [24]. The animals were maintained under a 14:10-h light-dark cycle at 24 ± 2°C and 50 ± 10% humidity. They were housed in plastic cages and allowed free access to tap water and normal chow (F-2, Funabashi Farm, Funabashi, Japan). Care for the animals and all experimental procedures were approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University.

Electrode implants

At 10 weeks of age, the rats were anesthetized by intraperitoneal injection of 45 mg/kg pentobarbital. A bipolar electrode was implanted into the basolateral amygdala to record EEG and permit stimulation, according to the rat brain atlas. The coordinates for the implant were 2.2 mm posterior and 4.8 mm lateral to the bregma, and 8.5 mm deep from the skull surface [12]. Each bipolar electrode consisted of two insulated stainless steel wires that were each 0.1 mm in diameter. Their tips were separated from each other by 0.5 mm. Three stainless steel screws were positioned over the frontal, parietal, and occipital cortices. The frontal and parietal screws were used to produce monopolar EEG recordings, and the occipital screw was used as a ground. The electrodes were connected to plugs and were held in place with dental resin that had been applied to the exposed surface of the skull.

Determining pre-kindling ADT and inducing kindling

Two weeks after implanting the electrodes, afterdischarge (AD) was induced using a 60 Hz monophasic square-wave pulse for one second (Fig. 1A). Stimulation began at 25 μA and was increased in a stepwise manner by 25 μA for each step. Each stimulus was carried out at ten-min intervals. ADT is defined as the lowest current (μA) that produces AD with a duration of at least five seconds as illustrated in Fig. 1B [11]. From the next day, 500 μA was constantly delivered to the amygdala at one-day intervals as seen in Fig. 1A. The severity of each seizure was classified by behavior according to Racine's criteria [14]. For all stimuli, the seizure severity and the ADD were recorded. The ADD was counted as the total duration of the amygdala EEG spikes of amplitudes of at least twice that of the pre-stimulus recording. When a subsequent AD was evoked within 10 seconds of the

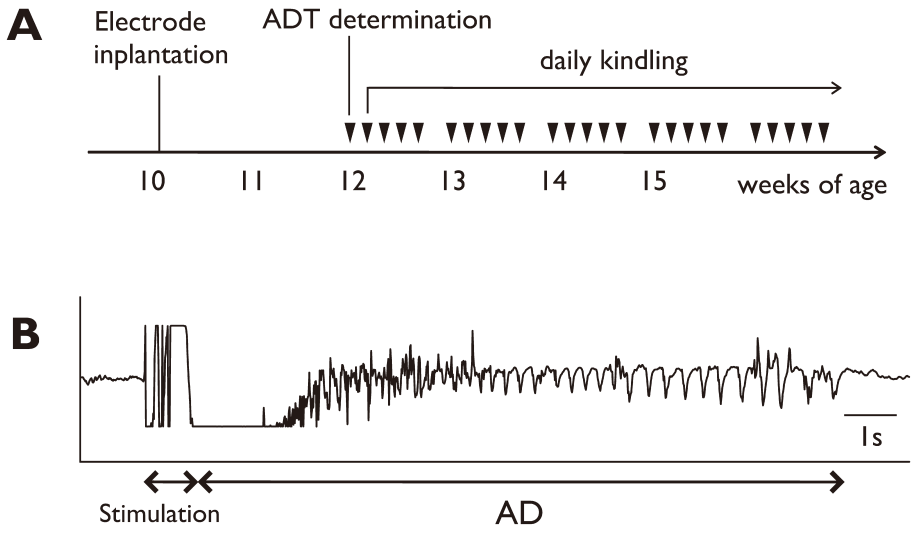

Fig.1. Experimental schedule and representative EEG trace of afterdischarge. (A) Schedule for inducing kindling. At 10 weeks of age, each animal had an electrode implanted into its amygdala. At 12 weeks of age, the animals were subjected to pre-kindling ADT determination as indicated by the open arrowhead. The next day, daily kindling stimuli were applied to the animals as noted by the arrowhead to determine the ADD. (B) A representative EEG recoding of the AD. This AD lasted at least 13 seconds. Just after stimulation, the amplitude of the EEG was too great to be recorded.

initial AD, the Ads were regarded as being one AD. By observing microscopically 8-μm-thick cresyl violet-stained coronal sections of the kindled rat brain, we confirmed that the stimulation electrodes had been placed on the amygdala.

QTL analysis

QTL analysis for the kindling rate was performed using 1,033 SNP markers [16] and WinQTL Cartographer Version 2.5. Composite interval mapping was employed as the QTL mapping strategy. The logarithm (base 10) of the odds (LOD) score was calculated to evaluate the significance of linkage. The LOD threshold was determined by 10,000 genome-wide permutations for each trait. The significance level was $P<0.001$.

Statistical analyses

Significant differences between the parental strains were identified using the Student's *t*-test. Analysis of variance (ANOVA) followed by Tukey-Kramer test post hoc comparisons were used to assess any differences between the strains.

Results

No significant differences existed for the pre-kindling ADT and ADD

The LE/Stm strain had a significantly lower ADT than did the F344/Stm strain: 108 ± 11 vs 170 ± 21 μA (mean ± SEM) ($P<0.05$). The ADTs of the seven RI strains varied within the range of their parental strains, as illustrated in Fig. 2A. The results of evaluating the data of the nine strains by ANOVA did not indicate that any significant differences existed in their ADTs ($P=0.138$).

The ADD recorded for the ADT currents of the parental strains was 7.3 ± 2.0 s for the LE/Stm strain and 10.7 ± 1.3 s for the F344/Stm strain, as shown in Fig. 2B. No significant differences were observed ($P=0.084$). Similarly, the ADDs of the nine strains varied from 7.1 ± 1.8 s for the LEXF2B/Stm strain to 10.7 ± 1.3 s for the F344/Stm strain, as shown in Fig. 2B. ANOVA of the data also indicated no significant differences were present in the ADDs that were recorded at the ADT among the nine strains ($P=0.51$).

Significant differences were observed for the kindling rate

For the parental strains, the kindling rates were 3.9 ±

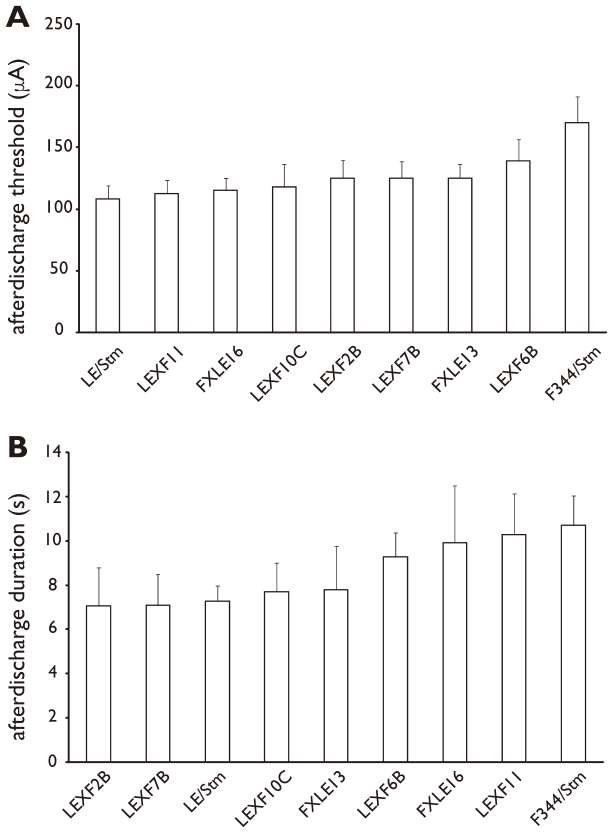

Fig. 2. Pre-kindling ADT of the LEXF/FXLE RI and parental strains. (A) Pre-kindling ADTs of the seven RI and two parental strains. There were no significant differences in the ADTs among the strains. (B) The ADDs of the seven RI and two parental strains, which were obtained when the pre-kindling ADTs were determined. There were no significant differences in the ADDs among the strains. The data are shown as the mean ± SEM.

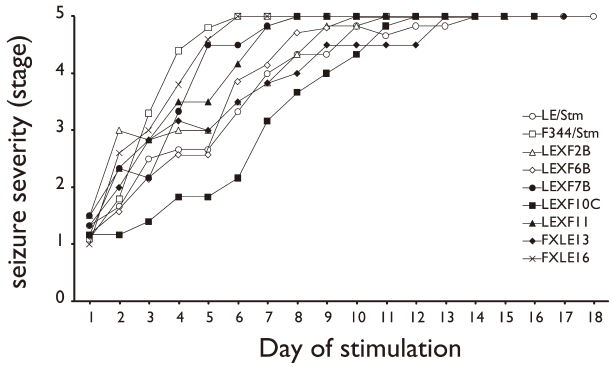

Fig. 3. Kindling development of the LEXF/FXLE RI and parental strains. (A) Average of the seizure severity stage that was observed during daily kindling stimulation for the RI and parental strains. The seizure sensitivity stage was classified as follows by behavior according to Racine's criteria: stage 1, mouth and facial twitches; stage 2, clonic head movements; stage 3, forelimb clonus; stage 4, clonic rearing; stage 5, loss of postural control (falling) [14].

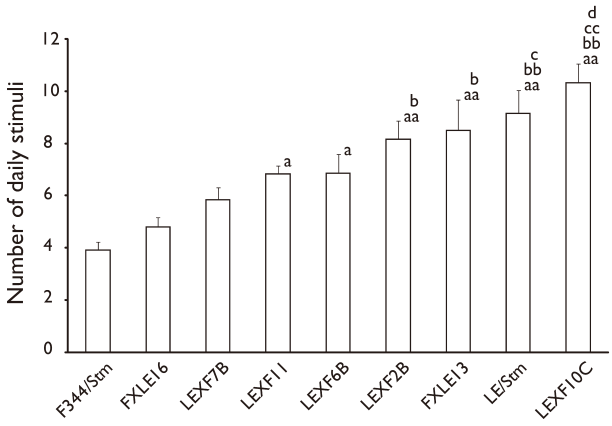

Fig. 4. Kindling rates of the LEXF/FXLE RI and parental strains. The kindling rate was defined by the number of daily stimuli that were applied until the first stage 5 seizure. Rat strains that received a smaller number of stimuli were more susceptible to kindling development, while rat strains that received a larger number of stimuli were more resistant to kindling development. Strain F344/Stm showed a significant difference in the number of stimuli that it received compared with strains LEXF11, LEXF6B, LEXF2B, FXLE13, LE/Stm, and LEXF10C ($^{a}P<0.05$; $^{aa}P<0.01$). Strain FXLE16 showed a significant difference in the number of stimuli that it received compared with strains LEXF2B, FXLE13, LE/Stm, and LEXF10C ($^{b}P<0.05$; $^{bb}P<0.01$). Strain LEXF7B showed a significant difference in the number of stimuli it received compared with strains LE/Stm and LEXF10C ($^{c}P<0.05$; $^{cc}P<0.01$). Strains LEXF11 and LEXF6B showed significant differences in the number of stimuli that they received compared with strain LEXF10C ($^{d}P<0.01$). The data are shown as means ± SEM.

0.3 for the F344/Stm strain and 9.2 ± 0.9 for the LE/Stm strain, as depicted in Figs. 3 and 4. The LE/Stm strain showed significantly lower kindling development than did the F344/Stm strain ($P<0.01$). Compared with the number of stimuli in each stage, differences between the parental strains were detected in stage 3 ($P<0.05$) and stage 4 ($P<0.01$) (data not shown).

For almost all of the nine strains, stage 5 was reached between three and 13 days for all rats, as seen in Fig. 3. Some rats showed particularly rapid kindling and skipped some stages, mainly stage 3. Others exhibited unstable kindling in where sometimes the stage was lowered.

The kindling rate varied from 3.9 ± 0.3 for the F344/Stm strain to 10.3 ± 0.7 for the LEXF10C/Stm strain, as illustrated in Fig. 4. ANOVA revealed a significant difference between the strains in the kindling rate ($P<0.01$).

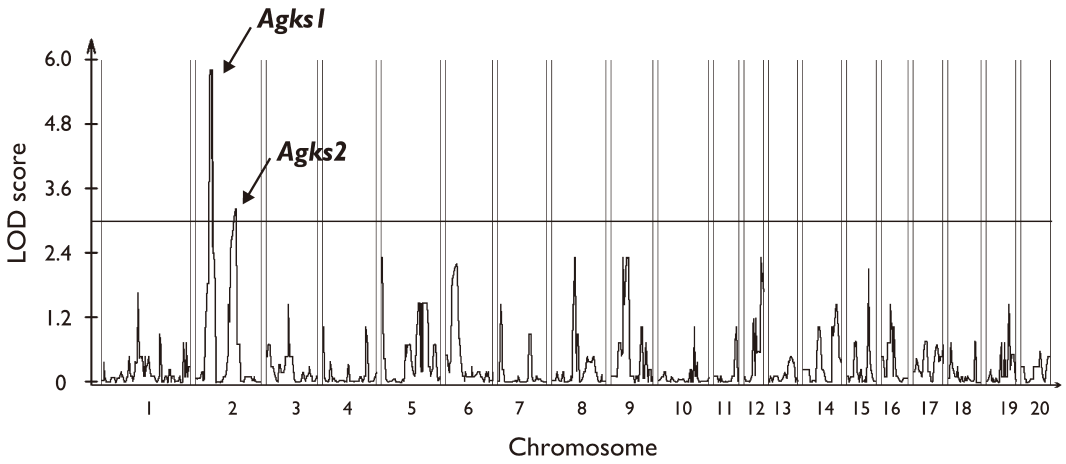

Fig. 5. Significant QTLs for the kindling rate. LOD score profiles of the kindling rate for rat chromosomes that were obtained for the seven RI and parental strains. The level of significance (LOD score was 3.0) is indicated by the horizontal solid line. Two QTLs, loci *Agkr1* and *Agkr2*, were detected on Chr 2, as indicated by the arrows.

Table 1. Alleles in loci *Agkr1* and *Agkr2*

QTL[1]	Marker	Position (Mb)	F344/ Stm	FXLE16	LEXF7B	LEXF11	LEXF6B	LEXF2B	FXLE13	LE/Stm	LEXF10C
Agkr1	J1264377	39.0	F[2]	F	F	F	F	L	L	L	F
	J521613	39.6	F	F	F	F	F	L	L	L	L
	J696978	48.8	F	F	F	L	F	L	L	L	L
Agkr2	Cpn_2146719424	146.7	F	F	L	L	F	F	F	L	L
	J561921	152.9	F	F	L	L	L	F	F	L	L
	gnl|ti|896632170_ 19866867240019_297	164.9	F	F	L	L	F	F	F	L	L

[1]Both QTLs are located on Chr 2. [2]F, homozygous for F344/Stm allele; L, homozygous for LE/Stm allele.

Post hoc analysis revealed stepwise differences strain-by-strain, as illustrated in Fig. 4.

Kindling susceptibility loci were identified by QTL analyses

QTL mapping for the kindling rate was performed on the nine strains. Analyses with WinQTL Cartographer revealed two significant QTL peaks for Chr 2, as shown in Fig. 5. These were named amygdala kindling rate 1 (*Agkr1*) and 2 (*Agkr2*). The locus *Agkr1* was defined by the proximal marker, J1264377 (39.0 Mb), and the distal marker, J696978 (48.8 Mb). The highest LOD score was 5.8 at the marker J521613 (39.6 Mb). The J521613 SNP marker showed a good association with the kindling rate. The F allele of J521613 seemed to be associated with fast development of kindling, while the L allele seemed to be associated with slow development of kindling. The locus *Agkr2* was defined by the proximal marker, Cpn_2146719424 (146.7 Mb), and the distal marker, gn l|ti|896632170_19866867240019_297 (164.9 Mb). The highest LOD score was 3.2 at the marker J561921 (152.9 Mb) (Table 1).

Discussion

The aim of this study was to identify the QTLs that are responsible for susceptibility to amygdala kindling and from that the genetic components that are involved in kindling development. A rat LEXF/FXLE RI strain panel that has been widely used for QTL analysis [19, 24] was employed.

The characteristics of seven RI and two parental strains for amygdala kindling-related parameters (ADD, ADT, and kindling rate) were evaluated. No differences

in the ADD or the ADT were observed among these strains, which was partly explained by the small numbers of animals examined. Meanwhile, significant inter-strain differences were found in the kindling rate. Furthermore, two QTLs (*Agkr1* and *Agkr2*) were identified for the kindling rate on rat chromosome 2 (Chr 2). The kindling rate, the number of daily stimuli until the first stage 5 seizure occurs, is thought to be indicative of kindling development, which is the progressive development of partial seizures into a more complex form [5]. Therefore, the findings of this study clearly show that genetic components are involved in kindling development in rats and that they entail several major genes and not multiple minor genes.

The rat is a valuable model of human neurological diseases, such as epilepsy and neurodegenerative disease [1, 19]. The concept that genetic factors play important roles in progressive kindling development could be applied to explain epileptogenesis in human epilepsy. It is reasonable to consider that some patients with partial seizures may be predisposed to generalized seizures and/or complex partial seizures. Indeed, Kobayashi *et al.* studied the outcomes of 98 patients in 22 unrelated families that are afflicted with mesial temporal epilepsy. They noted with respect to their seizures the existence of a strong genetic component. This component influenced the development of mesial temporal sclerosis in their families [6]. Of importance, in humans, most subjects that are examined in a clinical setting are those who have already developed seizures. Thus, the findings of this study are significant because substantial genetic factors may be difficult to identify based on clinically examining epileptic patients whose epileptogenesis has run its course.

Further study of kindling susceptible QTLs *Agkr1* and *Agkr2* would contribute towards identifying the genes that are responsible for kindling development. These genes should be associated with the progression and generalization of partial seizures and should be responsible for causing epileptogenesis. Additionally, their gene products could be good target molecules for developing new treatments, diagnoses, and ultimately preventing epilepsy. Within the *Agkr1* and *Agkr2* loci, 54 and 112 genes have been mapped, respectively. Among these, 23 within the *Agkr1* locus and 49 genes within the *Agkr2* locus are expressed in the brain [23]. Several sources in the literature have investigated genes that might be related to the kindling rate. In the *Agkr1* locus, the genes Polo-like kinase 2 (*Plk2*), heat shock protein-27 (*Hspb3*), and integrins (*Itga1*, *Itga2*) [3, 7, 18] are upregulated in epileptic animals. In the *Agkr2* locus, potassium voltage-gated channel beta member 1 (*Kcnab1*), which is associated with idiopathic epilepsy [17], is upregulated. These genes are choice candidates for investigation. Specifically, their expression levels in the brain before or after kindling and polymorphisms in these genes in LE/Stm and F344/Stm rats should be examined. Producing congenic strains carrying loci the *Agkr1* or *Agkr2* locus will permit narrowing down of these loci and help in evaluating their effects in identical genetic backgrounds.

In summary, this study revealed that substantial genetic components are involved in kindling development in the rat. The results suggest the existence of genes that underlie progression of epileptogenesis.

Acknowledgments

We thank the National BioResource Project–Rat for providing us with the F344/Stm, LE/Stm, and LEXF/FXLE RI strains. This work was supported in part by a Grant-in-aid for Cancer Research from the Ministry of Health, Labour and Welfare and Grants-in-aid for Scientific Research from the Japan Society for the Promotion of Science (18300141 and 21300153 to TK and 20240042 to TS).

References

1. Aitman, T.J., Critser, J.K., Cuppen, E., Dominiczak, A., Fernandez-Suarez, X.M., Flint, J., Gauguier, D., Geurts, A.M., Gould, M., Harris, P.C., Holmdahl, R., Hubner, N., Izsvak, Z., Jacob, H.J., Kuramoto, T., Kwitek, A.E., Marrone, A., Mashimo, T., Moreno, C., Mullins, J., Mullins, L., Olsson, T., Pravenec, M., Riley, L., Saar, K., Serikawa, T., Shull, J.D., Szpirer, C., Twigger, S.N., Voigt, B., and Worley, K. 2008. Progress and prospects in rat genetics: a community view. *Nat. Genet.* 40: 516–522. [Medline] [CrossRef]
2. Bailey, D.W. 1971. Recombinant-inbred strains. An aid to finding identity, linkage, and function of histocompatibility and other genes. *Transplantation* 11: 325–327. [Medline] [CrossRef]
3. Bidmon, H.J., Gorg, B., Palomero-Gallagher, N., Schliess, F., Gorji, A., Speckmann, E.J., and Zilles, K. 2005. Bilateral, vascular and perivascular glial upregulation of heat shock protein-27 after repeated epileptic seizures. *J. Chem. Neuroanat.* 30: 1–16. [Medline] [CrossRef]
4. Gilby, K.L., Da Silva, A.G., and McIntyre, D.C. 2005. Differential GABA(A) subunit expression following status epilepticus in seizure-prone and seizure-resistant rats: A putative mechanism for refractory drug response. *Epilepsia* 46

(Supple 5): 3–9.
5. Goddard, G.V., McIntyre, D.C., and Leech, C.K. 1969. A permanent change in brain function resulting from daily electrical stimulation. *Exp. Neurol.* 25: 295–330. [Medline] [CrossRef]
6. Kobayashi, E. Lopes-Cendes, I., Guerreiro, C.A., Sousa, S.C., Guerreiro, M.M., and Cendes, F. 2001. Seizure outcome and hippocampal atrophy in familial mesial temporal lobe epilepsy. *Neurology* 56: 166–172. [Medline] [CrossRef]
7. Lively, S. and Brown, I.R. 2008. Extracellular matrix protein SC1/hevin in the hippocampus following pilocarpine-induced status epilepticus. *J. Neurochem.* 107: 1335–1346. [Medline] [CrossRef]
8. Loscher, W., Cramer, S., and Ebert, U. 1998. Differences in kindling development in seven outbred and inbred rat strains. *Exp. Neurol.* 154: 551–559. [Medline] [CrossRef]
9. Lu, L.M., Shisa, H., Tanuma, J., and Hiai, H. 1999. Propylnitrosourea-induced T-lymphomas in LEXF RI strains of rats: genetic analysis. *Br. J. Cancer* 80: 855–861. [Medline] [CrossRef]
10. Mashimo, T., Voigt, B., Tsurumi, T., Naoi, K., Nakanishi, S., Yamasaki, K., Kuramoto, T., and Serikawa, T. 2006. A set of highly informative rat simple sequence length polymorphism (SSLP) markers and genetically defined rat strains. *BMC Genet.* 7: 19. [Medline] [CrossRef]
11. Mohapel, P., Zhang, X., Gillespie, G.W., Chlan-Fourney, J., Hannesson, D.K., Corley, S.M., Li, X.M., and Corcoran, M.E. 2001. Kindling of claustrum and insular cortex: comparison to perirhinal cortex in the rat. *Eur. J. Neurosci.* 13: 1501–1519. [Medline] [CrossRef]
12. Paxinos, G. and Watson, C. 2005. The rat brain in stereotaxic coordinates. 5th ed. Elsevier Academic Press, Amsterdam; Boston.
13. Plomin, R., McClearn, G.E., Goramaslak, G., and Neiderhiser, J.M. 1991. Use of recombinant inbred strains to detect quantitative trait loci associated with behavior. *Behav. Genet.* 21: 99–116. [Medline] [CrossRef]
14. Racine, R.J. 1972. Modification of seizure activity by electrical stimulation. I. After-discharge threshold. *Electroencephalogr. Clin. Neurophysiol.* 32: 269–279. [Medline] [CrossRef]
15. Racine, R.J., Steingart, M., and McIntyre, D.C. 1999. Development of kindling-prone and kindling-resistant rats: selective breeding and electrophysiological studies. *Epilepsy Res.* 35: 183–195. [Medline] [CrossRef]
16. Saar, K., Beck, A., Bihoreau, M.T., Birney, E., Brocklebank, D., Chen, Y., Cuppen, E., Demonchy, S., Dopazo, J., Flicek, P., Foglio, M., Fujiyama, A., Gut, I.G., Gauguier, D., Guigo, R., Guryev, V., Heinig, M., Hummel, O., Jahn, N., Klages, S., Kren, V., Kube, M., Kuhl, H., Kuramoto, T., Kuroki, Y., Lechner, D., Lee, Y.A., Lopez-Bigas, N., Lathrop, G.M., Mashimo, T., Medina, I., Mott, R., Patone, G., Perrier-Cornet, J.A., Platzer, M., Pravenec, M., Reinhardt, R., Sakaki, Y., Schilhabel, M., Schulz, H., Serikawa, T., Shikhagaie, M., Tatsumoto, S., Taudien, S., Toyoda, A., Voigt, B., Zelenika, D., Zimdahl, H., and Hubner, N. 2008. SNP and haplotype mapping for genetic analysis in the rat. *Nat. Genet.* 40: 560–566. [Medline] [CrossRef]
17. Schulte, U., Thumfart, J.O., Klocker, N., Sailer, C.A., Bildl, W., Biniossek, M., Dehn, D., Deller, T., Eble, S., Abbass, K., Wangler, T., Knaus, H.G., and Fakler, B. 2006. The epilepsy-linked Lgi1 protein assembles into presynaptic Kv1 channels and inhibits inactivation by Kvβeta1. *Neuron* 49: 697–706. [Medline] [CrossRef]
18. Seeburg, D.P. and Sheng, M. 2008. Activity-induced Polo-like kinase 2 is required for homeostatic plasticity of hippocampal neurons during epileptiform activity. *J. Neurosci.* 28: 6583–6591. [Medline] [CrossRef]
19. Serikawa, T., Mashimo, T., Takizawa, A., Okajima, R., Maedomari, N., Kumafuji, K., Tagami, F., Neoda, Y., Otsuki, M., Nakanishi, S., Yamasaki, K., Voigt, B., and Kuramoto, T. 2009. National BioResource Project-Rat and related activities. *Exp. Anim.* 58: 333–341. [Medline] [CrossRef]
20. Shin, R.S., Anisman, H., Merali, Z., and McIntyre, D.C. 2004. Amygdala amino acid and monoamine levels in genetically Fast and Slow kindling rat strains during massed amygdala kindling: a microdialysis study. *Eur. J. Neurosci.* 20: 185–194. [Medline] [CrossRef]
21. Shisa, H., Lu, L., Katoh, H., Kawarai, A., Tanuma, J., Matsushima, Y., and Hiai, H. 1997. The LEXF: a new set of rat recombinant inbred strains between LE/Stm and F344. *Mamm. Genome* 8: 324–327. [Medline] [CrossRef]
22. Tachibana, M., Lu, L., Hiai, H., Tamura, A., Matsushima, Y., and Shisa, H. 2006. Quantitative trait loci determining weight reduction of testes and pituitary by diethylstilbesterol in LEXF and FXLE recombinant inbred strain rats. *Exp. Anim.* 55: 91–95. [Medline] [CrossRef]
23. Twigger, S.N., Shimoyama, M., Bromberg, S., Kwitek, A.E., and Jacob, H.J. 2007. The Rat Genome Database, update 2007–easing the path from disease to data and back again. *Nucleic Acids Res.* 35: D658–D662. [Medline] [CrossRef]
24. Voigt, B., Kuramoto, T., Mashimo, T., Tsurumi, T., Sasaki, Y., Hokao, R., and Serikawa, T. 2008. Evaluation of LEXF/FXLE rat recombinant inbred strains for genetic dissection of complex traits. *Physiol. Genomics* 32: 335–342. [Medline]