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Spontaneous Epileptiform Seizures but Increased Resistance to Kindled Seizures in a Mutant Sprague–Dawley Rat (*mf/mf*)

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HERBERG L. J., I. C. ROSE, F. DAVISON, M. THOM, A. BECKETT AND F. SCARAVILLI. *Spontaneous epileptiform seizures but increased resistance to kindled seizures in a mutant Sprague–Dawley rat (mf/mf).* PHARMACOL BIOCHEM BEHAV **58**(4) 993–1001, 1997.—Approximately 30% of a breeding colony of Sprague–Dawley rats homozygous for an autosomal recessive mutation mf ("mutilated foot") associated with a peripheral sensory neuropathy have been found unexpectedly to suffer spontaneous epileptiform attacks. Seizures ranged from brief episodes of compulsive running to tonic–clonic convulsions lasting for up to 30 s, recurring at intervals of hours or days. EEG recordings during seizures showed high-voltage 8–10 Hz spike trains that abated over the ensuing 1–2 min. Interictal records were usually normal. Twice-daily kindling of the amygdala (200 μA sinewave for 1.0 s) was unexpectedly ineffective. Most of the rats that had suffered spontaneous seizures failed to develop kindled afterdischarges, even after 30 kindling stimulations. Other *mf* rats developed prolonged high-amplitude kindled afterdischarges that were arrested at stage 2 and failed to evolve into convulsive seizures. Hippocampal dentate granule cells of kindled *mf* rats, stained for zinc by Timm's method, showed significantly less mossy fibre sprouting than wild-type Sprague–Dawley rats after the same number of kindled afterdischarges. A minority of the *mf* rats tested (2 of 14) kindled normally. Auditory stimulation ($n = 23$) or stroboscopic flicker ($n = 14$) failed to elicit seizures or running fits in any *mf* rat. Peripheral neuropathy corresponding to that in the *mf* rat, with resistance to kindling and diminished mossy fibre sprouting, have also been reported in transgenic mice with defective $p75^{NGFR}$ neurotrophin receptors. A homologous genetic defect in the rat could account for most of the features of the *mf* phenotype. © 1997 Elsevier Science Inc.

Compulsive running Epilepsy Kindling Mutation Mutilated Foot NGF $p75^{NGFR}$ Sprouting Timm's stain

THERE are few reported instances of spontaneous seizures in the laboratory rat, unlike the dozen or so associated with distinct genetic errors in mice (9). Quasi-spontaneous seizures may develop in rats after repeated or prolonged stimulation of the brain (1,7,24,45), but these are not strictly spontaneous and may be a consequence of gross brain damage (1,7). Certain strains of rat have been shown to be especially sensitive to loud high-pitched sound to which they react with explosive 'running fits' or with full tonic–clonic seizures but the fits do not occur without stimulation (2,18,30), or they do so only rarely (13). More recently, absence-like episodes or tonic–clonic attacks, sometimes occurring unprovoked, have been described in a crossing of *zitter* and *tremor* rat mutants (53), and this double-mutant appears to provide the best documented example of spontaneous seizures in rats.

Thus, it seemed of considerable interest when several members of a mutant colony of rats (*mf/mf*; mutilated foot) maintained by the Neuropathology Department were observed to suffer sporadically from spontaneous epileptiform seizures, ranging from transient immobility or compulsive running, to full tonic–clonic convulsions. The *mf* phenotype (25) is due to an autosomal recessive mutation resulting in loss of peripheral sensory neurons in the dorsal root ganglia (DRG), leading eventually to ataxia and dystrophic extremities (hence its name) (10,55). Extensive CNS involvement or behavioural abnormalities have not been previously reported

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(25). The young adult has a healthy appearance and behaves normally despite sensory signs, reminiscent of type II hereditary sensory and autonomic neuropathy in man (HSAN II) (14) but without obvious autonomic impairment. Similar phenotypes presenting with dystrophic DRG and mutilated feet have more recently been described in two lines of transgenic mice (32,57), respectively, carrying targeted mutations of the gene encoding the high-affinity tyrosine kinase receptor (Trk) for nerve-growth factor (NGF) (33) or the gene for the nonselective $p75^{NGFR}$ receptor that binds NGF as well as brain-derived neurotrophic factor (BDNF) and several related neurotrophic factors (3,32). The $p75^{NGFR}$ (−/−) phenotype (32) is an especially close match: it is viable, fertile, and is free from the disabling disturbances seen with the trk gene deletion (57) and in other transgenic neurotrophin-defective strains (11,17,27, 28,35), or after treatment with NGF antiserum (26). However, direct demonstration of a $p75^{NGFR}$ deficit in the *mf* rat has not so far been reported.

We report an exploratory study of the *mf* rats' seizures, including daily observation and EEG examination of seizure-prone and seizure-resistant individuals, together with tests for susceptibility to seizures induced by auditory and sensory stimulation or by kindling stimulation through permanently implanted electrodes, and histological investigation of seizure-related changes in the brain.

METHOD

Subjects

Twisted bipolar stainless steel electrodes of 0.25 mm nominal diameter (Plastics One, Roanoke, VA) were implanted for kindling in six adult rats, 7–8 months old, weighing between 500 and 600 g that had been seen to experience seizures on at least two occasions by separate observers during the course of daily surveillance from birth onward, and in eight *mf* rats of similar age that had not been seen to suffer seizures. A history of seizures recorded by animal laboratory staff, and their subsequent confirmation during the experimental screening period, was assumed to identify a seizure-prone group (fitters) more susceptible to spontaneous seizures than rats in which seizures were not seen and which were classified as seizure-resistant (nonfitters). This classification was necessarily tentative but no member of the seizure-resistant group required reclassification during the course of the study. Electrodes were aimed at the central amygdala, with stereotaxic coordinates A −3.0, 5.0, 9.2 (44). Kindling in the two *mf* groups was assessed also in relation to other rat strains by comparison with data from nine genetically wild-type hooded rats (Bantin and Kingman type PVG) that formed the control group in a contemporaneous kindling study with similar treatment parameters (22). Adult wild-type Sprague–Dawley rats, including the ancestral strain from which the *mf* mutant was originally derived (25) were unfortunately not available during the period of the experiment. However, Sprague–Dawley rats have been reported to kindle somewhat faster than other strains of laboratory rat, including hooded rats (6,48); this difference is in the opposite direction to, and would not account for the results in the present study of the *mf* mutant.

All rats to be kindled were screened for audiogenic seizures and in most cases for photically induced seizures, before undergoing surgery. The number of *mf* rats tested for audiogenic seizures was further supplemented by nine unused littermates of the kindled rats. Validation of the apparatus and procedure for inducing audiogenic seizures was carried out with an unselected sample of 18 adult male hooded rats.

The *mf* mutant colony was maintained under UK Home Office project licence No. 70/02090 held by F. Scaravilli. Experimental procedures conformed to the provisions of project licence No. 70/03224 held by L. J. Herberg.

Screening for Stimulation-Induced Seizures

Audiogenic seizures. Each rat was placed in a glass-topped sealed ceramic chamber (550 × 350 × 220 mm high) containing a domestic 5-V doorbell that sounded with an intensity between 101.5 and 102.2 dB relative to standard pressure level, measured in different parts of the chamber on a Type 1405E digital sound level meter (RS Components Ltd, Corby, UK). The bell was sounded for a maximum of 30 s or until the rat responded with convulsions or explosive running. Negative tests were repeated not more than twice at intervals of not less than 48 h.

Photic stimulation took place in a transparent observation cage containing an overhead stroboscopic xenon lamp (Model 1214B, Dawes Instruments Ltd, UK) flashing at 5, 10, 20, or 30 Hz in four consecutive 10-s bouts. Rats retested after electrode implantation were connected to flexible leads and the EEG was recorded during stroboscopic stimulation.

Kindling stimulation commenced about 10 days after recovery from surgery. Stimulation took place in a transparent observation cage as in previous studies (23). Seizure intensity was graded on Racine's six-point scale: 0, no response; 1, facial movement; 2, head nodding; 3, forelimb clonus; 4, clonus plus rearing; 5, clonus, rearing and falling (47). The duration of limb clonus, if present, was timed by stopwatch. Electroencephalographic activity was recorded from the implanted electrodes shortly before stimulation and from about 1.5 s after the end of stimulation on a Lectromed Multitrace 2 recorder (Letchworth, Herts, UK). During application of the stimulating current, the amplifier input was short-circuited and the rat was connected to a constant-current fixed-duration sinewave source by operation of a manually operated two-way switch. Recording was continued after stimulation until seizure activity had subsided. Afterdischarge duration was defined as the duration of poststimulus EEG activity of more than twice baseline amplitude and faster than 1 Hz.

Afterdischarge thresholds were determined on the first day of kindling by administering an ascending series of single 1.0-s 50-Hz sine-wave trains at intervals of approximately 1 min. Current intensities were incremented by decilog (i.e., approximately 25%) steps between 10 and 250 μA until stimulation was followed by an afterdischarge. The final current was taken as the afterdischarge threshold, and the procedure was counted as a kindling session. All other kindling stimulations consisted of a single 1.0-s train at 200 μA; this current was several times higher than typical threshold levels encountered in previous studies under similar conditions [see (23)]. Stimulations were repeated twice a day on 6 days per week, and continued until a maximum of three stage 4 seizures or one stage 5 seizure had been elicited, with a minimum of 25 and a maximum of 30 stimulations.

The relative proportions of different groups responding to kindling stimulation were compared with a chi-squared test corrected for sample size, and seizure progress was examined with an analysis of variance for repeated measures, supplemented by Dunnett's or Tukey's test for differences between means.

Procedure

Breeding rats from the *mf* colony were screened for audiogenic and photically elicited seizures and kept in the labora-

tory under daily (8-h) observation in their home cages for several days to check for spontaneous convulsive seizures. Electrode implantation under halothane anaesthesia was followed by a further 10-day observation period during which selected rats were connected to the EEG via an overhead swivel lead, with the chart recorder set on stand-by. Each rat was classified as seizure-prone or seizure-resistant before the commencement of kindling. At the end of the experiment the rats were perfused with 4% paraformaldehyde under terminal anaesthesia.

Histology

The position of the electrode tips in kindled rats was verified on photographic enlargements of 50-μm frozen sections or on histological sections stained with haematoxylin and eosin.

Neuropathological assessment was carried out on brains of two fitter and two nonfitter *mf* rats and two wild-type Sprague–Dawleys. Brains for this purpose were fixed by perfusion with 4% paraformaldehyde and postfixed overnight. Paraffin embedded 7-μm coronal sections were then stained with haematoxylin and eosin, or immunostained with 1:400 anti-GFAP (glial fibrillary acidic protein) antibodies (Dako Ltd, UK).

Sprouting of hippocampal mossy fibres in kindled rats was assessed on brain sections obtained separately from five additional nonfitter *mf* rats and four wild-type Sprague–Dawley controls. Each group was given 12 kindling sessions (according to the protocols specified above) so as to be equated with respect to stimulation and time elapsed from the start of kindling (8) but not with respect to seizure stage (seizure activity could not be readily equated because kindled *mf* rats commonly failed to convulse). The rats were killed under terminal anaesthesia 5 days after the final kindling session and perfused with sodium sulphide solution followed by 4% paraformaldehyde. Brains were stained by Timm's method (56) to detect zinc-rich sprouting of hippocampal mossy fibres. The maximum density of mossy fibre sprouting from granule cells into the supragranular layer in the region of the dentate hilus was quantified blindly with respect to rat, by consensus of three observers, on a six-point scale modified from Cavazos and colleagues (8): 0, no sprouting fibres; 1, sparse; 2, scattered; 3, patchily confluent; 4, generally confluent; 5, densely confluent. Group sprouting scores were compared by a Mann–Whitney test for nonrelated samples.

Brains were also obtained from nonkindled fitting and nonkindled nonfitting *mf* rats (one each), stained by Timm's method and examined for sprouting.

RESULTS

Behavioural Monitoring

About one-third of the *mf* breeding colony, consisting of homozygous *mf/mf* males and females, were seen to suffer tonic–clonic convulsions. The typical interseizure interval, as extrapolated from a 330-h daytime sampling of 25 affected rats, was approximately 5–8 h. Seizures occurred without warning, varied markedly in pattern between rats, and were unlike the stereotyped facial twitching, head nodding, rearing and falling elicited sequentially in typical kindled seizures (47). The *mf* rat, sometimes after a brief period of eye and jaw movement and squealing, would typically roll onto its side with one or both hindlimbs extended or in continuous clonus, and with continued vocalisation. Recovery was rapid, after about 20–60 s, without the postictal irritability superimposed on behavioural depression commonly seen after kindled convulsions. Stereotyped rearing on the hindlimbs, with falling, was infrequent. Milder seizures seen in some rats consisted only of episodes of rapid tight circling with a hopping gait and continuous vocalisation, lasting 30 to 60 s. Seizures occurred only in adults, none having been recorded in females under 10 weeks of age or males under 12 weeks, although rats of this age generally constituted about one-quarter of the colony (husbandry records, Ms Wendy Rooke).

Electroencephalography

Attempts to capture spontaneous seizures in progress met with limited success because attacks were unpredictable in onset, infrequent, and relatively brief. However, a seizure resembling a typical spontaneous seizure, marked by collapse, flexor spasm, hindleg clonus, and squealing, that developed unexpectedly at the end of a kindled stage 1 afterdischarge was recorded as an 8-Hz spike train lasting approximately 25 s (Fig. 1A). (This rat failed to develop kindled convulsions in any preceding or subsequent kindling session). Spontaneous seizures were also captured in several other rats, in recordings that commenced after the onset of convulsions. The recordings again showed high-amplitude spike trains, followed by isolated spikes that abated over the next 1–3 min (Fig. 1B).

In a few rats, random isolated spikes, a common interictal feature of amygdaloid kindling, were present throughout the later kindling sessions (Fig. 4A). The presence or absence of these spikes did not appear to be predictive of spontaneous seizures or of susceptibility to kindling.

Kindling

Nonepileptic rats. Repeated kindling of putatively seizure-free *mf* rats ($n = 8$) led initially to a progressive increase in afterdischarge duration (Fig. 2B), clonus duration (Fig. 2C), and seizure severity (Fig. 2A), but progress to full convulsive seizures was significantly slower than ordinarily seen in genetically wild-type hooded rats under similar conditions [strain vs. stimulations interaction, $F(15, 240) = 6.56$, $p < 0.001$, for kindling sessions 1–16] (Fig. 2A). Kindling was slow because most of the *mf* nonfitters seemed to develop local afterdischarges in a normal way (Fig. 2B) but then stalled at Racine stage 2 (head nodding). The ensuing transition to stage 3 then

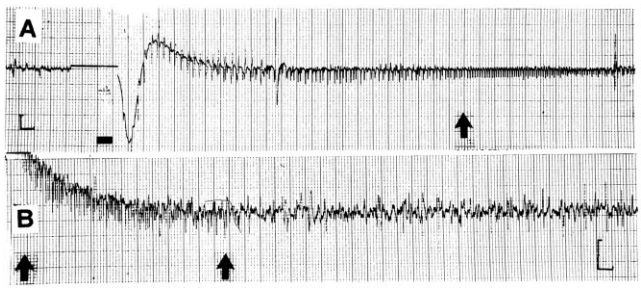

FIG. 1. Seizures in *mf* rats. (A) Kindled stage 1 seizure (eye-closure, and a 2.5-Hz spike-and-wave afterdischarge) followed (at arrow) by a 7–8 Hz spike train and a tonic–clonic seizure. The tonic–clonic seizure resembled the spontaneous seizures seen in this rat at other times, and was unlikely to have been a kindled seizure because this rat never progressed beyond stage 1 in 30 kindling sessions. Solid bar indicates kindling stimulation. (B) EEG recorded from amygdala towards the end of a spontaneous tonic–clonic seizure. A train of 7-Hz spikes (between arrows) gave way to intermittent spiking that abated gradually over the ensuing 5 min. Calibration: 1.0 s and 100 μV.

required many more stimulations than the transitions between other stages, $F(3, 23) = 15.86$, $p < 0.001$ (Fig. 3). Prolonged high-intensity afterdischarges evoked in the amygdala of these rats were typical in appearance (e.g., the afterdischarge recorded in Fig. 4A) but did not lead to generalised convulsions, in some cases even after 30 kindling sessions.

Two other *mf* rats in this group were successfully kindled at a more rapid rate, comparable to that usually seen in genetically normal rats.

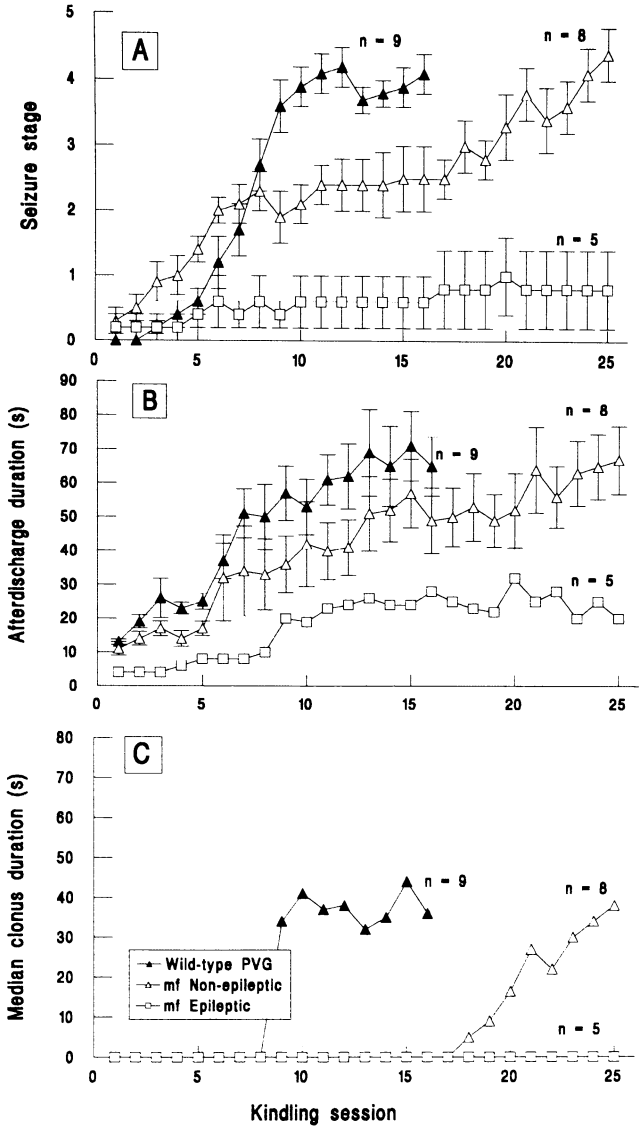

FIG. 2. Seizure stage (A), afterdischarge duration (B), and median duration of forelimb clonus (C) at successive kindling sessions in five *mf* rats with spontaneous fits, and in eight *mf* nonfitters. The upper curve (filled triangles) in each panel shows results from nine genetically wild-type hooded rats subjected to the same kindling procedure in an unrelated contemporaneous study (16 kindling sessions only) (22). Seizure 1 on day 1 was elicited by stimulation at threshold intensity. All subsequent kindling stimuli were suprathreshold (200 μA). Error bars indicate standard errors of the mean. They are omitted from the lower curve in panel B because more than half the spontaneous fitters failed to develop kindled afterdischarges and gave zero scores.

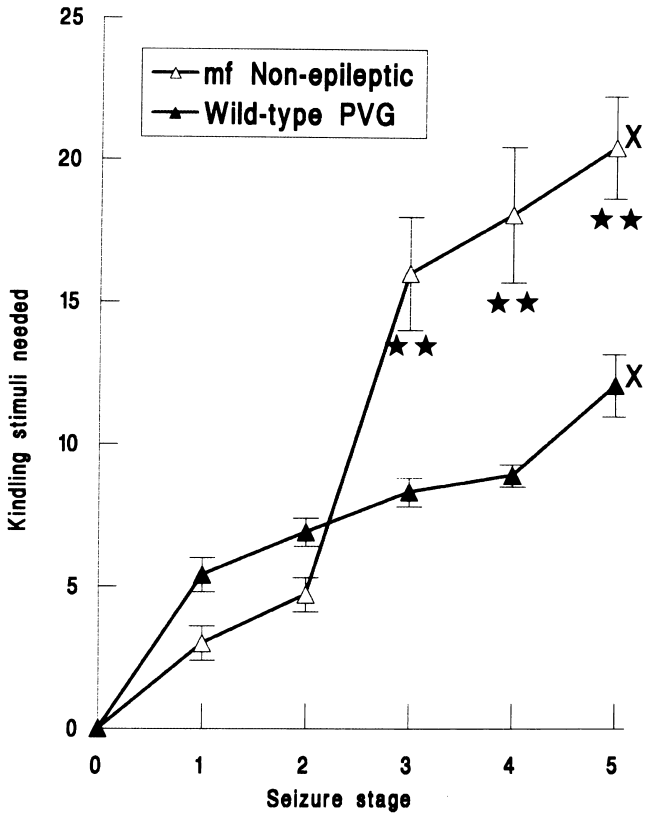

FIG. 3. Mean number of kindling stimulations (±SE) needed to reach successive seizure stages by nonepileptic *mf* rats and by genetically wild-type hooded rats. The evenly progressive curve for the hooded rats [data from (22)] is typical of results from wild-type laboratory rats, and differs significantly from the curve for the *mf* rats, $F(4, 68) = 13.2$; $p < 0.01$. The sharp inflection in the curve for *mf* rats between stages 2 and 3 reflects the disproportionately large number of kindling stimulations that had to be administered before local afterdischarges elicited in the amygdala spread to other brain areas. *Significantly different from hooded rats [Tukey (a) $p < 0.01$]. *Data for stage 5 omit one rat (in each group) that failed to progress beyond stage 4.

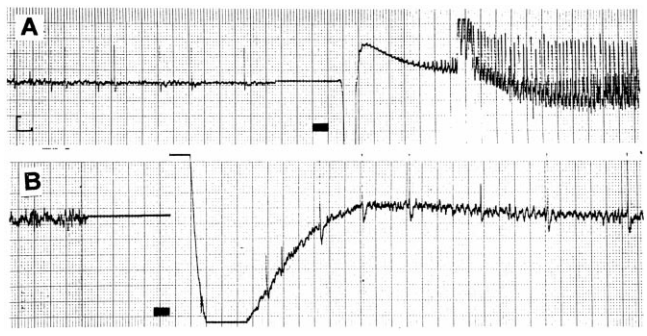

FIG. 4. (A) Pre- and postkindling record of a nonepileptic *mf* rat showing intermittent high-amplitude interictal spikes, interrupted, after a kindling stimulus, by a 3–5 Hz afterdischarge and a stage 2 kindled seizure (head nodding). (B) Unusual high-amplitude spikes elicited by kindling stimulation in a spontaneous fitter. This rat, and most of the other spontaneous fitters, failed to develop kindled afterdischarges or seizures after 30 kindling stimulations. Solid bars indicate kindling stimuli. Calibration: 1.0 s and 100 μV.

Epileptic rats. Four of six *mf* fitters failed to develop kindled afterdischarges after stimulation at intensities up to 250 μA, and a threshold could not be determined in these rats. One of the rats was excluded from further experiment (because of suspected infection) but 30 twice-daily kindling stimulations in the remaining fitters, at maximal intensity (250 μA), failed to elicit afterdischarges or behavioural signs of seizure activity. In some fitters, kindling stimulation was followed by an unusual pattern of irregular high-amplitude spikes (Fig. 4B) with a frequency and duration (typically 5 to 15 spikes over a period of minutes) that did not resemble kindled afterdischarges. Two fitters developed typical afterdischarges but did not progress beyond stage 2 and stage 3, respectively, after 30 kindling sessions. Their median afterdischarge threshold (80 μA) was not appreciably different from that of nonfitters (72 μA), but the proportion of fitters that developed afterdischarges (within the space of 30 kindling sessions) was less than for the nonfitting *mf* rats, and there was a significant association between the observed presence of spontaneous seizures and subsequent failure to develop kindled afterdischarges, $\chi^2(1) = 7.47$, $p < 0.01$. Examination of salvaged electrodes confirmed electrode continuity and insulation.

Sensory stimulation

Sounding of the doorbell in a preliminary validation study produced prompt and unambiguous running fits in 50% of 18 wild-type hooded control rats on their first exposure, including four running fits that evolved into a generalized tonic seizure. In 23 *mf* rats, however, the doorbell produced a vigorous startle reaction, suggestive of relatively normal auditory acuity, but no compulsive running or other signs of seizure activity were seen in the 30 s during which stimulation was administered.

Stroboscopic stimulation failed to promote seizure activity in either the *mf* rat or in wild-type hooded rats, including nine hooded rats known to be susceptible to audiogenic seizures. Nonspecific mechanical disturbance or handling of *mf* rats in the course of routine husbandry was similarly without observed effect.

Histology

Electrode location. Kindling electrodes, including electrodes that failed to elicit afterdischarges, were located within the anterior or basal nucleus of the amygdala, or, in two rats (both of which were successfully kindled), in the immediately adjacent endopyriform cortex (Fig. 5).

Neuropathological assessment. Brains of *mf* rats (Figs. 6A and B) revealed a variable degree of ventricular dilatation but the cortical ribbon showed a normal six-layered distribution of nerve cells without dysplastic features. GFAP staining showed discrete gliosis, predominantly in the fifth and sixth cortical layers and in the subjacent white matter (not shown). No evidence of an inflammatory process was seen. The hippocampus, including the dentate and the levels of the cornu ammonis, showed normal morphology, though GFAP staining indicated a moderate uniform increase in glial cells. The brains of *mf* fitters (Fig. 6B) showed an overall reduction in size when compared to those of normal wild-type Sprague–Dawley rats (Fig. 6C).

Kindling-induced sprouting. Kindling electrodes in brains to be examined by Timm's method were located in the lateral basal (seven rats) or medial basal (two rats) amygdaloid complex (not shown). Sprouting hippocampal mossy fibres in the region of the dentate hilus were detected 5 days after the 12th kindling session, both in *mf* rats [median sprouting score (8) = 2.0, range 0–3] (Fig. 7A), as well as in the wild-type Sprague–Dawley group [median = 4.5, range 2–5] (Fig. 7B). However, the scores for sprouting in *mf* rats were significantly lower than in wild-type controls (Mann–Whitney U (4,5) = 2.5, $p < 0.05$).

In nonkindled rats, Timm's procedure showed very few stained fibres crossing from dentate granule cells into the stratum oriens, and there was no appreciable difference among nonkindled fitting and nonfitting *mf* rats and nonkindled normal rats (Fig. 7C).

DISCUSSION

The laboratory rat is widely used for the study of experimental seizures, and the *mf* mutant may prove useful for studies of seizures that occur spontaneously [cf. (42)]. A surprising feature of the *mf* rat was its resistance to audiogenic seizures. This finding sets it apart from the Genetically Epilepsy Prone (GEP) rat (30) of which the salient feature is extreme hypersensitivity to auditory stimulation. Equally unexpected was the resistance to kindling shown by most of the *mf* rats. The *mf* rats' resistance to kindling contrasts with the abnormally rapid kindling reported in the GEP rat (54).

These disparate features, spontaneous seizures coupled with heightened resistance to experimental seizures, form a puzzling combination: it is hard to envisage a disorder that could be responsible for both. Several possibilities can be excluded as unlikely on present evidence. It might be suggested, for example, that each spontaneous seizure could have produced a postictal rise in seizure threshold that prevented the occurrence of kindled or audiogenic seizures. However, postconvulsive refractoriness in the rat, after a single convulsive seizure, from whatever cause, has usually been found to decline over a period of approximately 2 h (24,41,52,59) and would not be expected to confer protection unless the rat had suffered overt convulsions in the preceding 2 h. The usual interval between spontaneous convulsive seizures in the *mf* rat was much longer than this.

Other, more long-lasting postictal disturbances that could affect kindling in the *mf* rat include the occurrence of status epilepticus or closely repeated convulsions that may destroy epileptic substrate in amygdala and hippocampus (5,37) or substantia nigra (34). This may result in a paradoxical resistance to further seizures (5,37,38). Frequent closely repeated seizures may also be followed by continuous abnormal 3-Hz activity ('minor status'), with a similar consequence (15,23). However, the spontaneous seizures of the *mf* rat were not particularly severe or closely repeated, and histological and electroencephalographic findings in the present study did not disclose the specific lesions such as have been reported to affect kindling in this way (5,37,38). Kindling at any particular electrode site may also be antagonised by prior kindled seizures at a different site (4,20), but this phenomenon appears to depend on a gating action by prior kindling (to which the intact *mf* fitter was not exposed), rather than on prior seizures (4). On the contrary, a history of previous seizures, as in the *mf* rat, could well lead one to expect a significant saving in the number of stimulations needed to elicit kindled convulsions (23). A further possibility is that glutamatergic systems necessary for kindling and for audiogenic seizures (12,39,40,43) could have been compromised by excitotoxic glutamatergic autoantibodies (51,64) developed against the *mf* rats' peripheral lesions (10,55). Crossreactive antibodies to subunits of brain glutamate receptors are known to be apt to give rise to intractable seizures (51). However, no inflammatory reaction

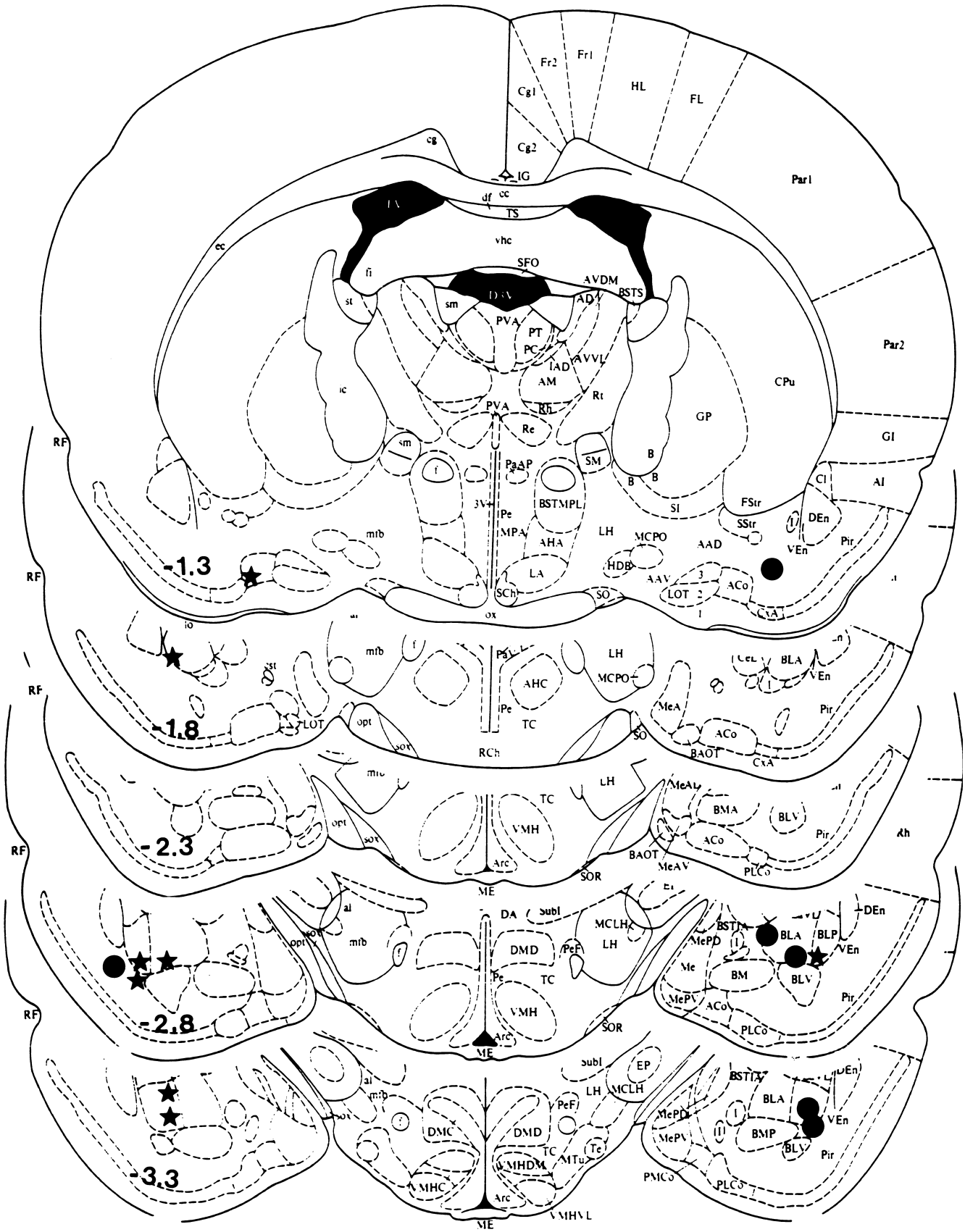

FIG. 5. Electrode sites represented on coronal sections of rat brain at 0.5-mm intervals, adapted with permission from the atlas of Paxinos and Watson (43). Spontaneous fitters are plotted on the right-hand half of the sections, nonfitters are on the left. Filled circles signify rats that did not develop kindled afterdischarges, or did not progress beyond stage 2 (head nodding) even after 30 kindling sessions. Stars signify rats that were eventually kindled to at least stage 3 (forelimb clonus). Numerals on left indicate a-p coordinates relative to bregma.

suggestive of an autoimmune reaction to glutamate receptors (64) or immunoreaction to any other brain constituent was detected in the brain sections examined.

The most prominent neuropathological feature of the *mf* rat is their shrunken DRG (10)—an improbable cause of seizures. Atrophic DRG are, however, a strong pointer to underlying neurotrophin dysfunction, their most usual heritable cause (17,26,27,32,57,58). Neurotrophins are also known to serve as neuromodulators regulating neuronal plasticity in adult brain (46,63) and to be involved in kindling. For example, hippocampal NGF and BDNF mRNAs show sharp rises in kindled subjects (16,21) accompanied by sprouting of dentate granule cell mossy fibres (50,60,62), and both effects may be necessary for successful kindling [(19,29,49,65) but cf. (31). On the other hand, BDNF (+/−) knockout mice with only a half-normal BDNF response are delayed at the same stage of kindling (stage 2) (29) as were most of the *mf* rats in the present study. Kindling is also delayed in genetically wild-type rats treated with anti-NGF serum, although in this case the delay was found to be most marked either at stage 0 (19) (as we found in spontaneously fitting *mf* rats), or else all stages were delayed (65). Thus, the peripheral neuropathy of the *mf* rat, its resistance to kindling, and the consequent reduction in hippocampal sprouting in the Timm-stained sections could be parsimoniously accounted for by neurotrophin dysfunction, and in particular, by defective $p75^{NGFR}$ receptor activity.

The occurrence of spontaneous seizures in the *mf* rat is less easy to account for. Dysfunctional regrowth and reorganization of hippocampal mossy fibres has been implicated in the development of spontaneous temporal lobe seizures in man

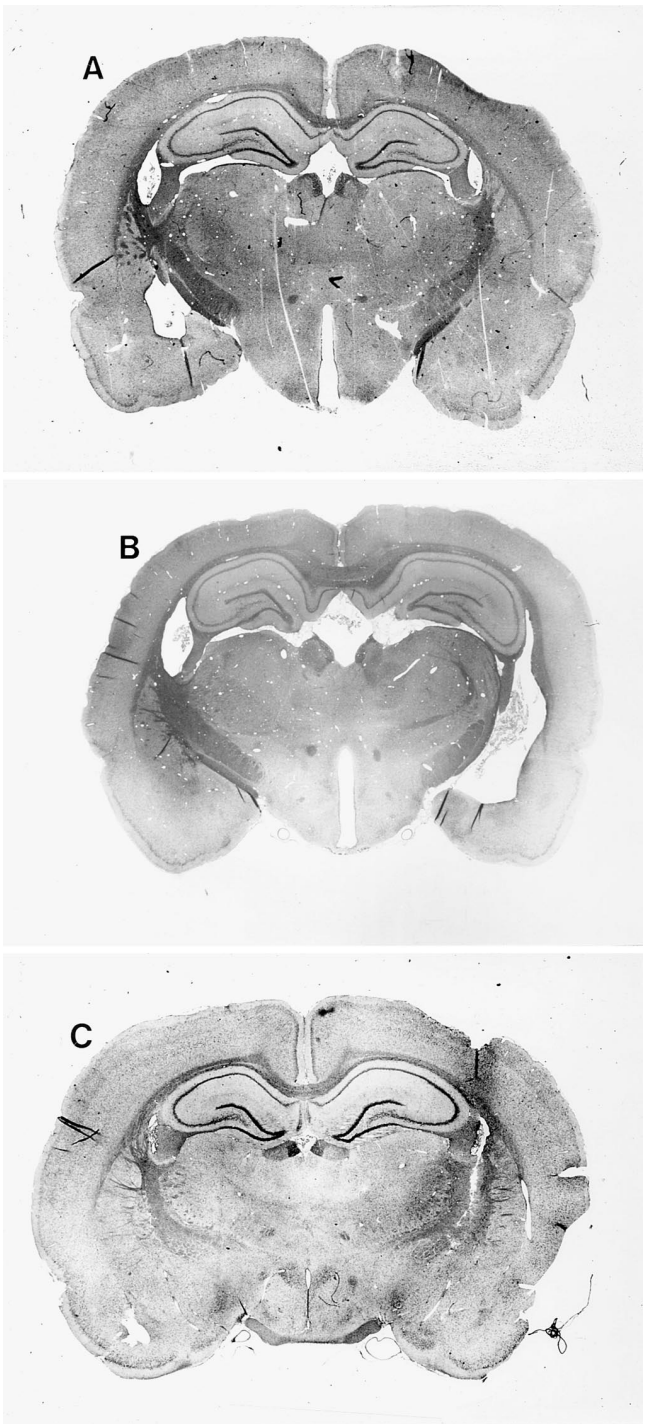

FIG. 6. Histology of *mf* rat brain. Coronal sections of brain stained with haematoxylin and eosin, ×8: (A) nonfitting *mf* mutant. (B) *mf* mutant that had suffered repeated spontaneous seizures, showing dilated ventricles overall and reduction in brain size. (C) Normal brain of wild-type Sprague–Dawley rat.

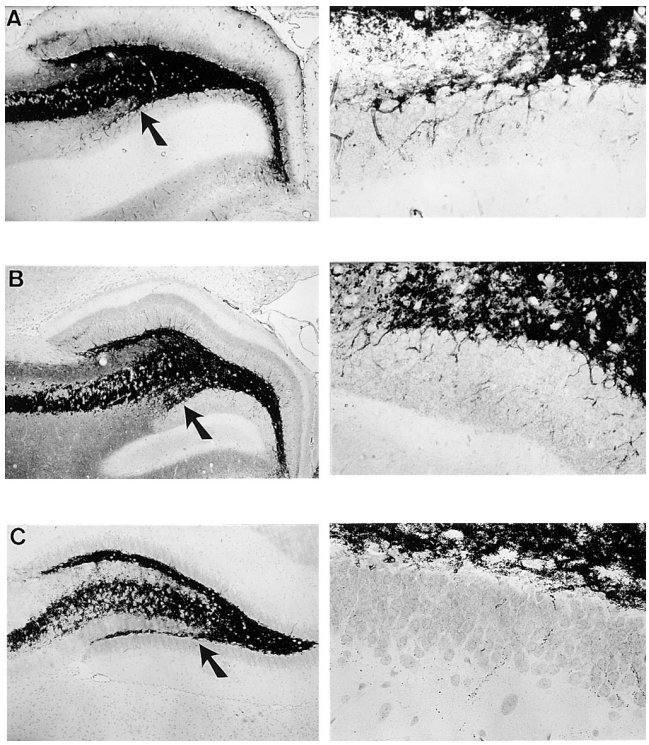

FIG. 7. Sprouting of hippocampal mossy fibres stained by Timm's method. Low power (×16) and high-power (×75) coronal sections through hilus of hippocampal dentate. (A) Wild-type Sprague–Dawley killed 5 days after the 12th kindling stimulus, showing relatively intense (grade 5) mossy fibres sprouting from dentate granule cells into supragranular molecular layer; (B) Kindled *mf* rat, showing grade 3 sprouting; (C) Nonkindled wild-type Sprague–Dawley showing virtual absence of sprouting mossy fibres. The sections shown were from the rat in each group that showed the most sprouting.

(36,61) and kindled seizures in rats (50,62), but the spontaneous seizures of the *mf* rat are unlikely to have arisen in this way. Neurotrophin deficits are more likely to inhibit aberrant sprouting than to provoke it [(65), but see (29)]. No overgrowth of mossy fibres was revealed by Timm's method in nonkindled *mf* rats in the present study. The patterning and EEG of the *mf* rats' spontaneous seizures (see introductory section) are moreover unlike those of limbic seizures typically associated with hippocampal mossy fibre sprouting. The spontaneous seizures of the *mf* rat seem more likely to originate from foci elsewhere in the brain, whether as a consequence of other central effects of the *mf* mutation [e.g., (66)], or from an associated genetic defect.

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