Pharmacology Biochemistry and Behavior, Vol. 43, pp. 1081–1086, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Severity of Experimental Allergic Encephalomyelitis in Rats Depends Upon the Temporal Contiguity Between Limbic Seizures and Inoculation

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Received 7 October 1991

MISSAGHI, B., P. M. RICHARDS AND M. A. PERSINGER. *Severity of experimental allergic encephalomyelitis in rats depends upon the temporal contiguity between limbic seizures and inoculation.* PHARMACOL BIOCHEM BEHAV 43(4) 1081–1086, 1992.—Limbic seizures (forepaw clonus) were induced in Lewis rats by subcutaneous injections of lithium (3 mEq/kg) followed 24 h later by a muscarinic agent. Either 7 days before, 7 days after, or on the day of seizures, rats were inoculated with a spinal cord preparation. Other groups received these preparations but served as treatment (no seizure) controls. In three separate experiments, rats in which seizures had been induced at the time of inoculation displayed significant increases in the severity of clinical symptoms 14–20 days later relative to controls while rats in which seizures had been induced 7 days after inoculation displayed less severe symptoms; the latter effect was partially simulated by multiple injections of 1 mg/kg dexamethasone. The immunofacilitation effect was much stronger than the immunosuppression and explained 25–30% of the variance in the clinical severity scores.

Limbic seizures Lithium Muscarinic effects Rats Experimental allergic encephalomyelitis Immunofacilitation Dexamethasone

CLINICAL and experimental observations have suggested a modulatory role of the CNS upon immunologic phenomena (1). The central role of the entorhinal (parahippocampal) cortices-hippocampal-amygdala complex and one of their major efferents, the hypothalamus, has been strongly implicated for both humoral and cellular immunity (3,5,13). Destruction of the anterior hypothalamus (5,8,11,12) inhibited anaphylaxis and delayed hypersensitivity to tuberculin, antibody synthesis, and experimental allergic encephalomyelitis (EAE), an experimental model for multiple sclerosis (2).

Because of the intricate influence of the mesiobasal temporal lobe structures upon descending hypothalamic pathways and the release of corticotropin (6), sustained periods of electrical seizures would be expected to affect the immunologic process. Such limbic seizures, with or without clinical correlates, could be sequelae to some closed head injuries or could be induced by strong emotional states. Recent work by Falter et al. (3) suggests that antibody formation (humoral immunity) displays an initial decrease (+5 days) and then a compensatory increase (+10 days) following induction of limbic seizures by subcutaneous injections of lithium (equivalent to clinical blood levels in human patients) followed 4–24 h later by the systemic introduction of a muscarinic agonist (9,13). In the following experiments, we examined the immunofacilitative and immunosuppressive effects of these types of seizures upon the development of EAE.

METHOD

Rats

In four separate experiments, a total of 117 60- to 80-day-old male and female Lewis rats were selected as subjects; they had been obtained from Charles River Breeding Farms (Hull, Quebec). All rats were housed in groups of three rats per cage and maintained in a temperature-regulated (20°C) and automatically determined light-dark (12 L : 12 D) environment. Purina rat chow and food were available ad lib.

Treatment

In Experiment I, male rats were assigned to one of four groups (eight rats/group). Three of the groups were injected subcutaneously with 3 mEq/kg lithium and then 30 mg/kg

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pilocarpine 24 h later either 7 days before, 7 days after, or on the same day as inoculation for EAE. The combination of the lithium-pilocarpine evoked motoric stereotypes that developed from mouth automatisms to forepaw clonus (7,9); the pattern closely resembles amygdaloid kindling. The blatant, sustained limbic seizures were verified for all animals.

On the inoculation day, both hind foot pads of each rat were injected with a total of 0.2 cc of a complete Freund's adjuvant that contained emulsified spinal cord of Lewis rats (8 mg injected per rat). A fourth (reference) group was injected with the spinal cord suspension but did not receive the seizure-inducing treatment. To facilitate survival of the rats, acepromazine (25 mg/kg) was injected subcutaneously into the neck region 1 h after seizure onset (9,13); overt signs of status epilepticus were eliminated within 1 h. This volume, delay time, and drug (acepromazine) were employed in all subsequent experiments.

In Experiment II, male rats were assigned to one of three groups: control(n = 13), limbic seizure +7 days after inoculation (n = 10), and limbic seizure on the day of inoculation (n = 8). The last two groups received the treatment given in Experiment I. To enhance the immunofacilitory component of the response, the inoculation dosage of spinal cord was 2 mg/rat; it was injected in volumes of 0.1 cc into each footpad (0.2 cc total).

In Experiment III, male rats were assigned to one of four groups: the first three groups, n = 8, 9, and 9, respectively, were the same as in Experiment II. A fourth group (n = 6) was introduced to control for the 10–15% weight loss that occurs during the 10 days that follow seizure induction. Each of these rats were yoked with one of the rats from the group in which seizures were induced on the day of inoculation. Each rat was weighed daily; food availability for group 4 was controlled so that a matched weight loss (within 2%) was maintained. The amount of spinal cord injected was 4 mg/rat (0.2 cc, both hind footpads).

In Experiment IV, female rats were assigned to one of three groups after inoculation with spinal cord emulsions that were similar to Experiment III. Seven days later, seizures were induced in the same manner by lithium and pilocarpine in one group (n = 8). Between 6 and 8 days after inoculation, a second group (n = 7) received two SC injections of 1 mg/kg dexamethasone (11) per day (separated by 8 h). The third group (n = 7) served as nontreated controls. All other housing and maintenance procedures were similar to the other experiments.

Rats were evaluated daily, beginning 9 days after inoculation, for clinical signs. A severity scale ranging from 0–5 was employed: normal, 0; loss of tail tonicity, 1; ataxia, 2; early paralysis, 3; complete paralysis of both hind legs, 4; and complete hind leg paralysis plus loss of bladder control and severe akinesia, 5. To complete the assessment, each rat was taken from the home cage and placed on a nearby table. Even though the clinical signs were unambiguous, reliability procedures were incorporated into the study. The correlation between independent raters for the mean impairment scores was

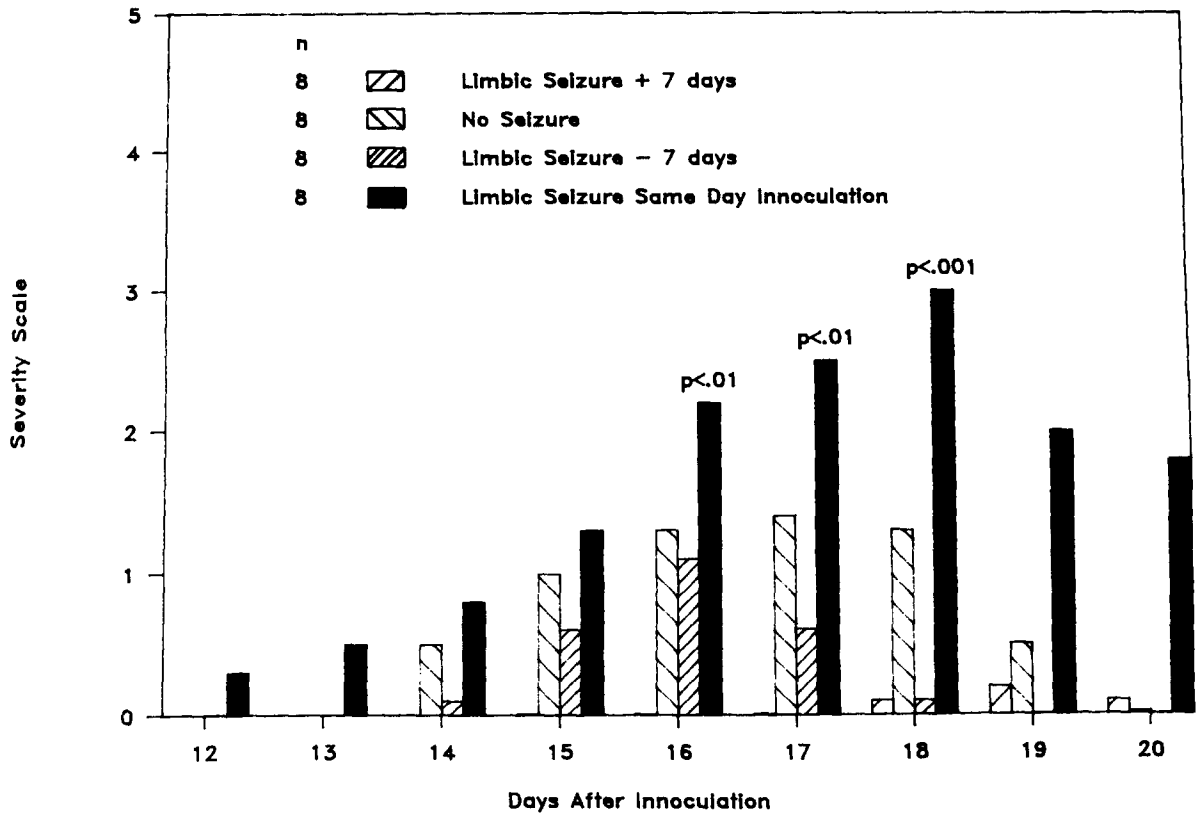

FIG. 1. Clinical severity of EAE as a function of days since inoculation for groups of rats in which limbic seizures had been induced by systemic lithium-pilocarpine either 7 days before, 7 days after, or on the day of inoculation. A nonseizured (but inoculated) reference group is also shown.

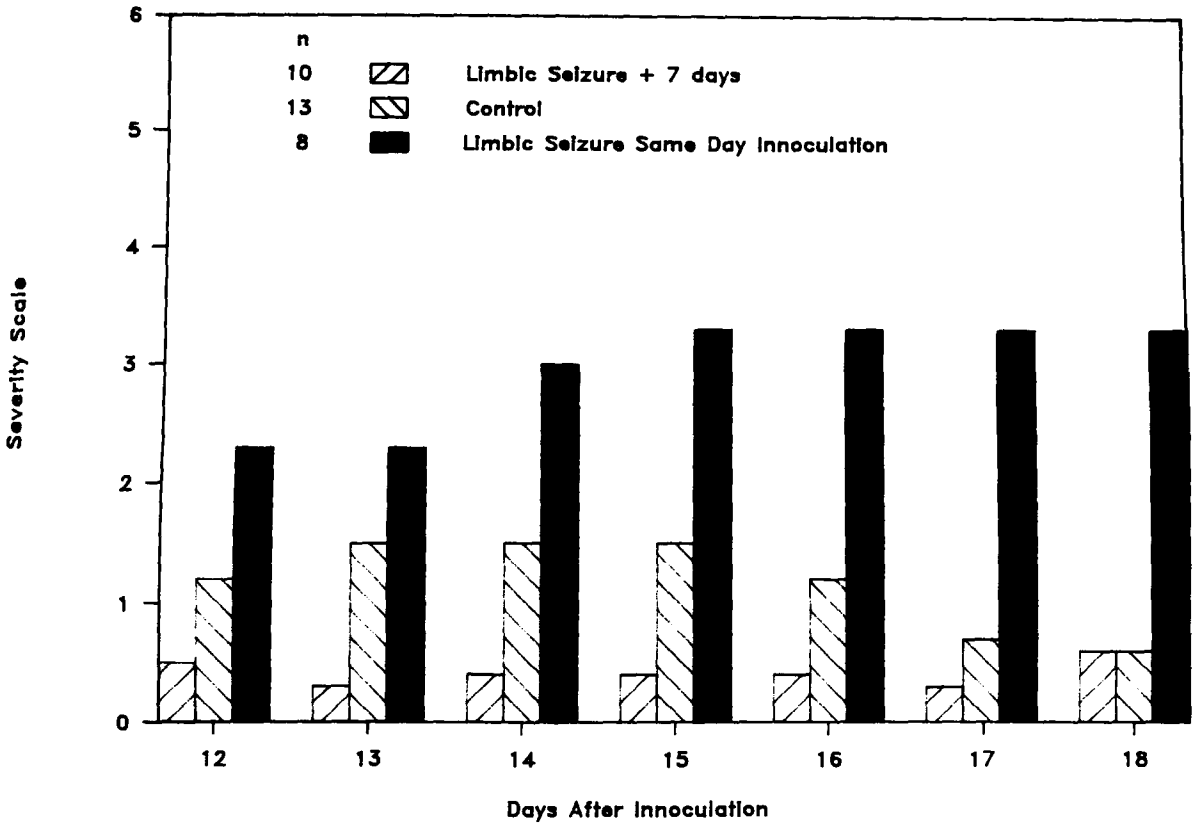

FIG. 2. Clinical severity of EAE as a function of days since inoculation for groups of rats in which limbic seizures had been induced the day of or 7 days after inoculation; a nonseizured (but inoculated) reference group is also shown.

0.92 in Experiment II. In Experiment III, rats were evaluated under blind conditions.

For each experiment, a series of one-way analyses of variance (ANOVAs), beginning on postinoculation day 12, were completed by SPSS^X software on a VAX 4000 computer. Posthoc comparisons employed Scheffe's set at $p < 0.05$. Because of the heterogeneity of variance between groups in some experiments, nonparametric ANOVAs (Kruskal-Wallis) were also performed. In light of the specific hypothesis pursued in Experiment IV, a multivariate ANOVA (MANOVA) was completed to discern potential interactions between the rate of development of the symptoms (days) and the treatments.

RESULTS

Figure 1 displays the daily severity score for groups in which the limbic seizures had been induced 7 days before, the same day as, or 7 days after inoculation with spinal cord; the nonseizured, inoculated reference group is also shown. Statistically significant ($p < 0.01$) treatment differences were evident on postinoculation days 16–20, $F(3, 28) = 3.34$–13.36, $p < 0.05$. Posthoc tests indicated that rats in which the limbic seizures had been induced on the same day as inoculation displayed significantly greater clinical severity than nonseizured controls while the group in which seizures had been induced 7 days after inoculation displayed significantly less clinical severity between postinoculation days 16–18. The group that displayed the seizures 7 days before inoculation did not differ from the reference (control) group.

Experiment II (Fig. 2) replicated the results of Experiment I; there were statistically significant group differences, $F(2, 28) = 4.14$–11.02, $p < 0.05$. Rats in which limbic seizures had occurred on the day of inoculation displayed facilitation of EAE development and severity compared to controls between days 13 and 18. Again, rats in which the same type of seizure was induced, only 7 days after inoculation, displayed relative suppression; posthoc analyses indicated that this suppression was significant only for postinoculation days 13–15.

Experiment III (Fig. 3) again replicated the basic pattern; significant group differences occurred between postinoculation days 14 and 17, $F(3, 28) = 3.35$–6.67, $p < 0.05$; $\chi^2 = 2.09$–10.07, $p < 0.05$. Posthoc analyses (verified by nonparametric comparisons between pairs of groups) indicated that rats in which seizures had been induced on the same day as challenge with the spinal cord emulsion and Freund's adjuvant displayed more severe clinical symptoms than the nonseizured control group. The group in which seizures had been induced 7 days after inoculation and the group that served as yoked, food-deprived controls for rats that received the treatment and inoculation on the same day both displayed less severity relative to controls on days 15–17 only. The mean scores for this period displayed statistically significant group ($F = 5.27$, $\chi^2 = 14.27$, $p < 0.01$) differences.

Subsequent analyses to determine the strength of the effect (Ω^2 estimates) indicated that treatments in each experiment accounted for between 35 and 45% of the variance in the severity scores between days 14 and 18. However, more than 75% of this variance was due exclusively to the enhanced

severity of symptoms displayed by the groups of rats in which the spinal cord had been injected and seizures induced on the same day. The strength of the immunosuppressive effect, defined as a decrease in the severity of the clinical symptoms, accommodated only about 20% of this variance and was always marginally significant from statistical criterion.

Experiment IV replicated the results of previous experiments and demonstrated that repeated dexamethasone treatments during the same period as induction of the limbic seizures only partially suppressed the severity and onset of EAE (Fig. 4). A two-way ANOVA with one level repeated (postinoculation days 12–18) indicated statistically significant differences between groups, $F(2, 19) = 11.98$, $p < 0.001$, and days, $F(6, 114) = 16.30$, $p < 0.001$; the group × day interaction was also statistically significant, $F(12, 114) = 3.48$, $p < 0.001$.

Single one-way ANOVAs indicated that the largest group differences occurred on postinoculation days 14 ($F = 8.28$, $\chi^2 = 10.33$), 15 ($F = 16.49$, $\chi^{2\blacktriangleleft} = 15.26$), and 16 ($F = 7.00$, $\chi^2 = 9.51$). Posthoc analyses indicated that although groups that received either the seizure or dexamethasome treatments displayed significantly less severity of EAE on postinoculation day 14 ($\chi^2 = 8.37, 4.87$, $p < 0.05$) the deficits of the latter group were comparable to the control group subsequent to postinoculation day 15. After day 19, there were no statistically significant differences in the severity of symptoms between any of the groups.

DISCUSSION

Our results consistently indicated three important features in the development of EAE in rats. First, systemic treatment with lithium (3 mEq/kg) followed 24 h later by 30 mg/kg pilocarpine evokes limbic seizures that can later influence the severity of EAE. Second, the *specific timing* of the seizure with respect to the time of inoculation determines if the severity of the EAE is less or greater than the reference group. Third, unlike the typical profile of immunosuppression produced by previous experimental manipulations of the limbic system (5,8,12), concurrent seizure induction was associated with a more pronounced immunofacilitation.

Although one could posit that seizure-induced alterations in metabolism or blood flow could have altered the clearance of the spinal cord suspension (to the footpad), this option is unlikely. Acepromazine, which was injected into rats in which seizures had been induced as well as controls, stabilizes heart rate to within normal levels and eliminates the overt myoclonus (Persinger, Harrigan, and Bureau, unpublished data). In addition, the possibility that acute changes in blood flow or metabolism, for a few hours either 7 days before or 7 days after inoculation, would influence clearance time of the suspension requires a relatively exotic mechanism.

The robust nature of the results is evident when its prevalence, despite the different parameters, is considered. Whereas the peak impairment scores in Experiment I and II for control

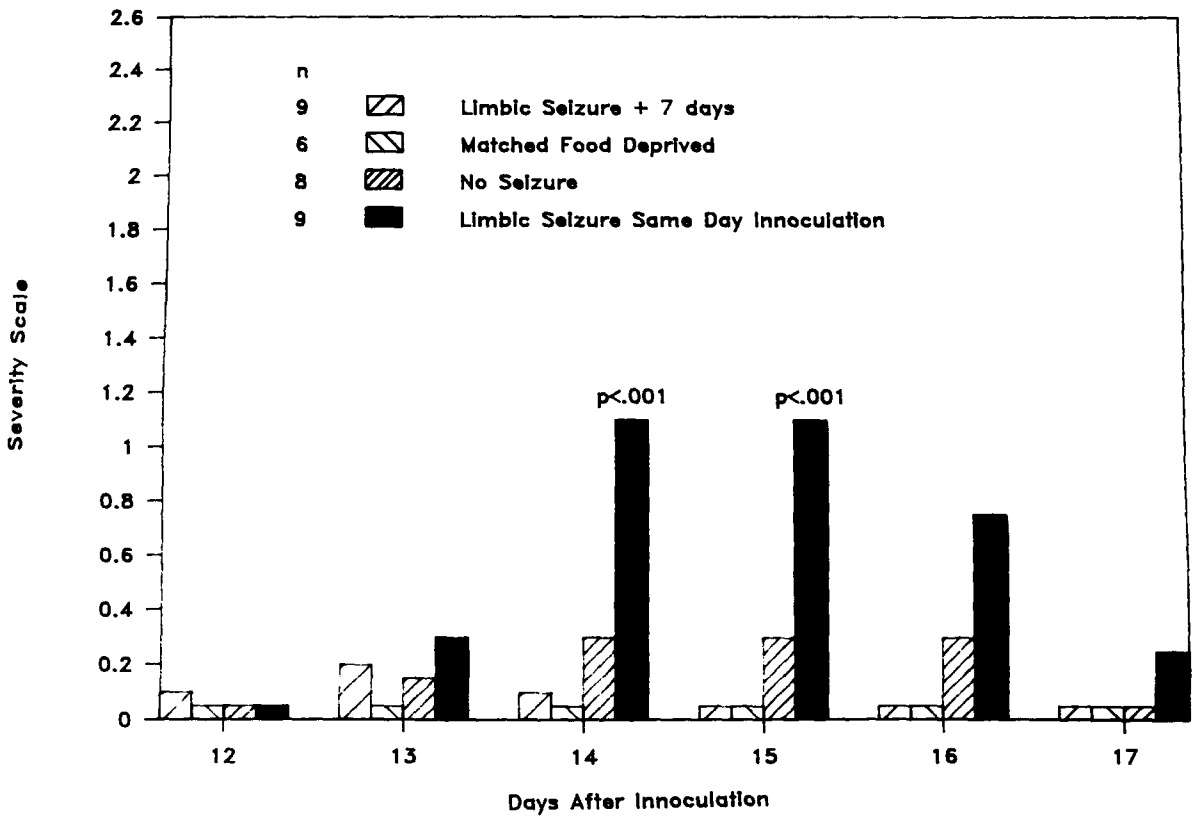

FIG. 3. Clinical severity of EAE as a function of days since inoculation for groups of rats in which limbic seizures had been induced the day of or 7 days after inoculation; nonseizured (but inoculated) and food-deprived (but inoculated) reference groups are also shown.

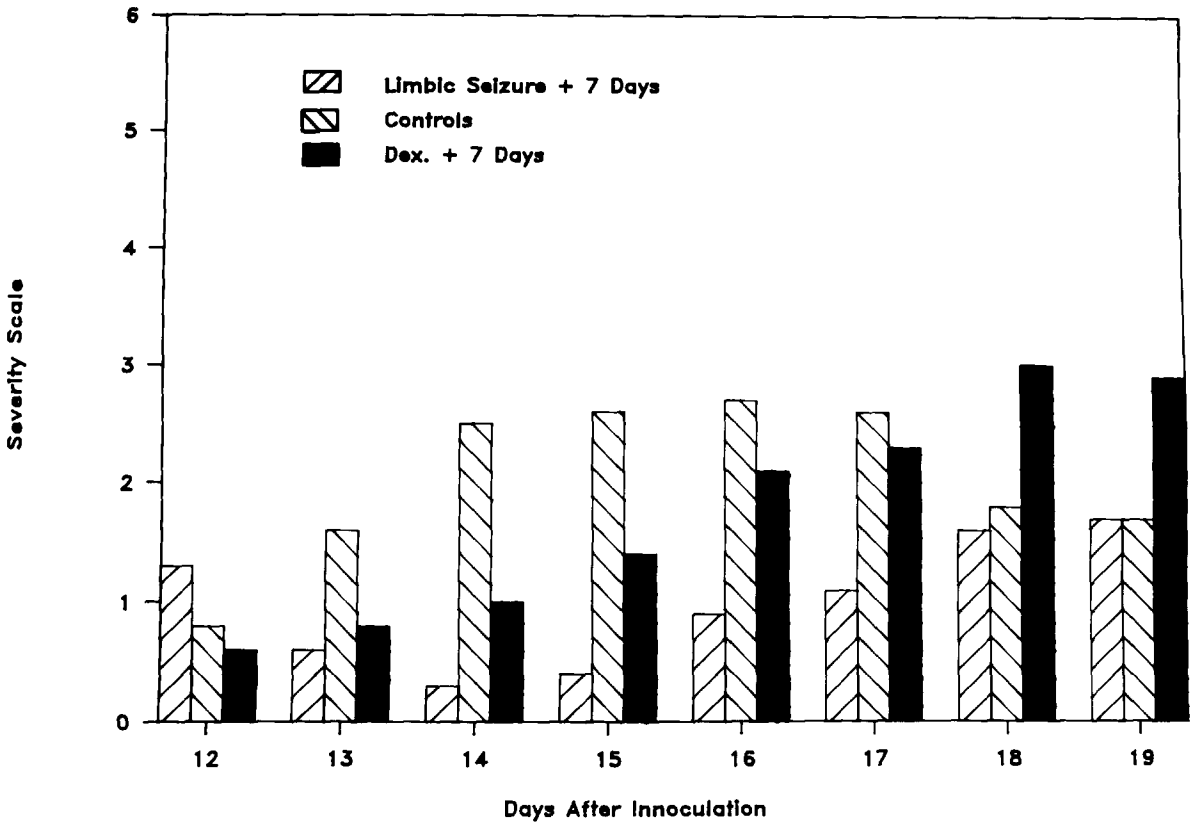

FIG. 4. Clinical severity of EAE as a function of days for groups of rats in which limbic seizures had been induced 7 days after inoculation or dexamethasone (Dex) had been injected 6–8 days after inoculation; controls (no seizure) are also shown.

rats were effectively identical, control rats in Experiment III displayed a marked attenuation in the development of EAE. However, this marked reduction would be commensurate with the maintained elevation of glucocorticoids or corticotropin that could have accompanied the daily handling of previously untouched animals. Finally, the marked elevation of EAE scores for controls in Experiment IV is expected in light of the well-known relative immunofacilitation of female rats (4) compared to male rats.

These results suggest that limbic seizures (despite differences in procedures) evoke temporally dependent modulations in the processes of cellular immunity, resulting in immunofacilitation or immunosuppression. Seizures evoked by the lithium-pilocarpine treatment have elevated corticotropin levels to maximum assay levels (× 10) within 1 h after onset; a fourfold elevation is usually evident 24 h later (4). Because corticotropin is an immunosuppressor, the occurrence of seizures 7 days after inoculation could easily delay or suppress the activated T-cell lymphocytes.

That this seizure-related suppression of EAE was in part mediated as well by glucocorticoids normally released by corticotropin is suggested by the partial simulation of the seizure effect (amelioration of the severity of symptoms) in rats that were given multiple injections of the powerful synthetic glucocorticoid dexamethasone. We are assuming that repeated injections did not also elicit corticotropin elevations. Until further studies are completed, we cannot determine if: a) our dosage [1 mg/kg; selected because of its effects upon initial seizure onset times (11)] was not sufficient to simulate the seizure-induced suppression, b) subclinical seizures and hence corticotropin/glucocorticoid release actually continue for periods of time that exceeded the window of dexamethasone injection, or c) both seizure-induced corticotropin and glucocorticoids mediate the EAE suppression.

The possible occurrence of immunofacilitation, as defined by both worsening and greater incidence of EAE for rats in which seizures were induced on the same day as inoculation, suggests that a rebound occurs about 1 week later and stimulates T-cell productivity; about 2 weeks after the seizure and inoculation, the intensified symptoms are clinically observed. Comparable effects have been reported in this seizure model for a humoral response (3). That the results of both the humoral and cellular immunity studies are related is suggested (Reid, Falter, and Persinger, unpublished data) by the enhanced antibody binding capacity (3) in rats 10 days after they have been administered the EAE procedure [n = 10, 282 (133) ng/mg] relative to controls [n = 15, 101 (58) ng/mg].

The scalar (magnitude) rather than vectorial (magnitude and time) change in the clinical symptoms suggests that seizures facilitated the size of the inflammatory component of the response rather than its development. The most parsimonious explanation would involve the normal daily fluctuations in circulating corticotropin. Bidirectional responses in the release of this neuropeptide, determined by the duration of stimulus-induced afterdischarges (ADs) within the mesiobasal structures of the temporal lobe, have been reported by Gallagher et al. in human patients (6). ADs of greater than 10 s enhanced corticotropin levels while ADs of less than 10 s reduced corticotropin to subnormal levels.

If this principle is valid within the present contexts, then

the maintained electrical lability (without overt seizure displays) within the entorhinal-amygdaloid region of the limbic lobe would be associated with ADs of less than 10 s during the week that follows seizure induction. During this period, circulating corticotropin levels would be decreased and immunofacilitation would be encouraged. We have not measured corticotropin during this period, but the decreased circulating corticosteroid levels (3) and reduced rate of weight gain (9) are not incompatible with this hypothesis.

The asymmetry of the severity of the clinical response does not support fluctuations in corticotropin as the total explanation for the variability in clinical severity. Optimally, one would expect that the absolute reduction in symptoms produced by putative elevation of corticotropin (immunosuppression) would be matched by a comparable absolute increase in the severity of the symptoms produced by the putative decrease in corticotropin (immunofacilitation). Clearly, however, the size of the immunofacilitation was much greater than the suppression.

This asymmetry could be an artifact of: a) scaling because the mean severity ratings for nonseizured reference groups were never within the mean (2.5) range and b) the consequences of mild immunofacilitation upon a system that is already immunofacilitated; we have found that antibody binding capacity for human serum albumin markedly increases as rats develop EAE, suggesting a systemic effect (Reid, Falter, and Persinger, unpublished data). If this explanation is correct, then the absolute effects of the seizures are similar but their impact and clinical consequences depend upon the state of the system.

Alternatively, the type of excitotoxic-induced brain damage in progress at the time of inoculation may exacerbate the clinical expression. In addition to damage within the pyriform-entorhinal cortices (9), rats exposed to the lithium-pilocarpine model for seizure induction also exhibit substantial necrosis and reactive gliosis within the substantia nigra (reticulata), dorsomedial thalamic nucleus, and centromedian-parafascicular complex of the thalamus.

Although this research has direct application to the development of multiple sclerosis, we think it may also have relevance to demyelinating processes that could follow mild closed head injuries. Direct and indirect indices of temporal lobe lability are frequently observed following traumatic brain insults. If they are associated with enhanced, even subclinical, electrical activity within the limbic system, then coincident exposure of the cellular immune system to myelin basic protein, due to concomitant microhemorrhaging or transient decrease in the efficacy of the blood-brain barrier, could increase the risk of subsequent demyelinating complications in susceptible patients.

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