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Opiate Modification of Amygdaloid-Kindled Seizures in Rats[1]

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STONE, W. S., C. E. EGGLETON AND R. F. BERMAN. *Opiate modification of amygdaloid-kindled seizures in rats.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 751–756, 1982.—Male Long-Evans rats were stereotaxically implanted bilaterally with bipolar electrodes in the central amygdala. Rats were then kindled once daily for 1 sec until 3 consecutive Stage V [25] kindled seizures were elicited. On the following day, animals were injected (IP) with either saline, naloxone (10 mg/kg), naltrexone (10 mg/kg) or morphine sulfate (10 mg/kg) and again stimulated at the kindling stimulation parameters. Saline injected animals continued to show long bilateral AD's and behaviors (i.e., forelimb clonus, rearing, falling) typical of Stage V kindled animals. In contrast, rats injected with naloxone or naltrexone showed reduced behavioral seizures. Potentiation of post-ictal spiking by morphine in amygdaloid-kindled rats was also observed supporting previous reports [7,21]. In a second experiment, the reduction of kindled seizure severity by naloxone was systematically replicated. It is concluded that opiates can significantly modify amygaloid-kindled seizures, and that brain endorphins may play a role in the development or maintenance of an amygdaloid-kindled seizure focus.

Kindling Amygdala Naloxone Naltrexone Morphine

RECENT attention has been directed towards the relationship between brain endorphin systems and the occurence of epileptiform activity. Earlier research demonstrated that morphine, either alone or in synergy with other convulsants, could promote seizures in mammals [18,38]. However, the mechanisms of action were largely undefined. The recent discovery of endogenous opiate receptors and ligands provided an important impetus to further examine the relationship, and several studies have now demonstrated that both exogenous and endogenous opiates are capable of producing EEG and behavioral manifestations of seizures [8–9, 34]. In addition, these studies have generally shown that such seizures are prevented or reversed by naloxone or other opiate antagonists.

Among the available paradigms for studying drug effects on seizures, the "kindling" model has been particularly useful. As described by Goddard [12], the procedure consists of repeated daily electrical stimulation of one of a number of specific brain areas. Initially, only local afterdischarges (AD) are observed. With repeated daily stimulation, the AD's increase in duration, propagate to interconnected neural systems and eventually result in generalized behavioral convulsions. The effect is permanent, and spontaneous seizures develop following extended kindling [26]. The technique provides a model for examining the nature of seizure development, and as an analog of epilepsy represents a useful screening model for anticonvulsants [1, 2, 36].

In the present study, the effects of opiates on amygdaloid-kindling were examined. A number of factors supported this selection. The amygdala is one of the most rapidly kindled areas of the brain [27]. It is also particularly interesting for a study of opiate effects as high concentrations of opiate receptors [19], enkephalinergic terminals [33], and enkephalins [16] have been identified there. Various opiate influenced or mediated behaviors, such as analgesia [10] and the morphine abstinence syndrome [22] have shown significant changes after amygdaloid-kindling. In addition, it has been reported that amygdaloid-kindled rats given morphine showed increased seizure severity and prolonged postictal spiking [21].

Although opiates appear to be implicated in seizure mechanisms, most prior attempts to modify kindled seizures with opiates have produced small or negative findings [1, 5, 7, 24]. In the present study, however, we report that under various stimulation parameters, opiate antagonists can significantly reduce seizure severity in Stage 5 amygdaloid-kindled rats, and morphine can significantly potentiate the severity of postictal events.

EXPERIMENT 1

METHOD

Subjects

Thirty-four adult male Long-Evans rats (300–350 g) were used. Animals were individually housed and fed ad lib. The animal colony was maintained on a 12:12 hr light-dark cycle.

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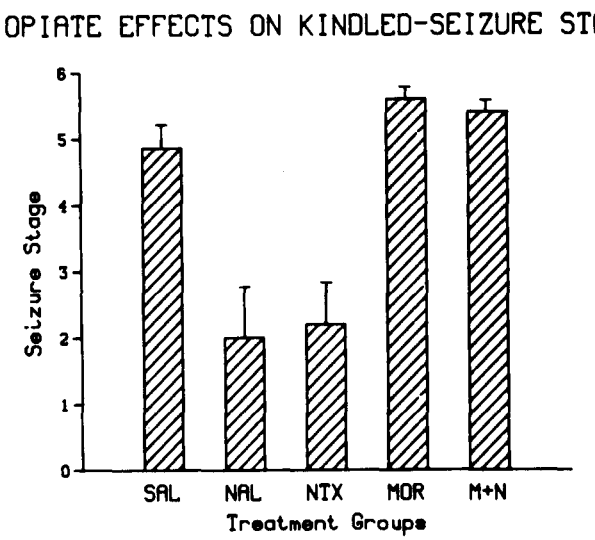

FIG. 1. Mean seizure stages of drug treated and saline control rats. Vertical lines on bars indicate standard deviations. (SAL—saline, n=7; NAL—naloxone, n=7; NTX—naltrexone, n=6; MOR—morphine, n=5; M+N—morphine and naloxone, n=6).

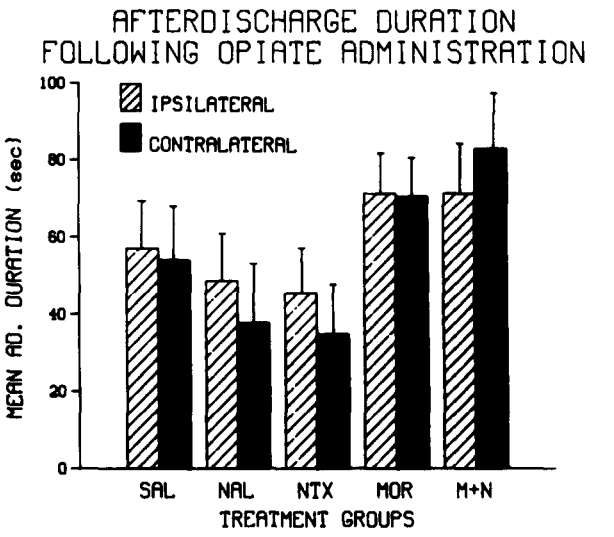

FIG. 2. Mean AD duration (sec) for ipsilateral (stimulated) and contralateral (recording only) sites for treated and control rats. Vertical lines on bars represent standard deviations. (SAL—saline, n=7; NAL—naloxone, n=7; NTX—naltrexone, n=6; MOR—morphine, n=5; M+N—morphine and naloxone, n=6).

Surgery

All rats were surgically anesthetized with sodium pentobarbital (60 mg/kg, IP), and stereotaxically implanted, bilaterally, with bipolar electrodes (Plastic Products MS/303, 0.25 mm outer diameter) aimed for the central amygdala. The electrode assembly was anchored to the skull with three stainless steel screws and dental acrylic. Animals were then allowed 1 week postsurgical recovery.

Afterdischarge Threshold

After recovery from surgery the electrical stimulation threshold for eliciting an afterdischarge was determined for each animal. Briefly, each rat was placed in a transparent Plexiglas recording chamber and leads from a Grass Model 7 polygraph were attached to the implanted electrodes. Five minutes of baseline EEG activity was recorded. Electrical stimulation was then delivered to one of the implanted electrodes via a Grass S88 stimulator equipped with two PSIU6 constant current isolation units. A switching device allowed rapid alternation between recording and stimulating through the electrodes. Stimulation intensity was monitored on a Tektronix 502A oscilloscope. The electrode used for kindling remained the same throughout the experiment, but bilateral recordings were obtained from all animals, both before and immediately after the period of stimulation.

One sec of electrical brain stimulation (EBS) was delivered beginning at a current intensity of 10 microamperes (100 Hz, 0.1 msec pulse duration, biphasic, symmetrical, rectangular pulses) and incremented by 10 microamperes until an afterdischarge (AD) was recorded. These stimulation parameters were chosen to produce reliable kindling without producing tissue injury [3]. The individual AD thresholds ranged between 70 and 500 microamperes.

Kindling

Rats were stimulated once daily at the AD threshold for 1 sec, until they reached the behavioral criterion of 3 consecutive Stage 5 seizures as definded according to the rating scale of Racine [26]. Behavioral ratings of later kindling stages (i.e., stages 6–8) were based on the scale of Pinel and Rovner [23]. This criterion was reached after 8–34 days (Mean=19.6, S.D.=15.4). The day after reaching criterion, animals were randomly assigned to groups and injected with either 0.9% saline (SAL, n=7), naloxone (NAL, 10 mg/kg, n=7), naltrexone (NTX, 10 mg/kg, n=6) or morphine (MOR, 10 mg/kg, n=6). Naloxone and naltrexone were administered 20 minutes before, and morphine 15 minutes before, EBS. A final group (M+N, n=8) was injected sequentially with morphine (10 mg/kg followed 15 minutes later with naloxone (10 mg/kg). Thus rats in Group M+N received naloxone 15

TABLE 1

OPIATE EFFECTS ON POSTICTAL SPIKING AND DEPRESSION

Groups	N	Spiking* (spikes/min)	% Showing spiking	Depression* (seconds)	% Showing depression
SAL	7	1.8 ± 1.6	86%	30.0 ± 2.7‡	71%
NAL	7	1.9 ± 2.0	71%	none	0%
NTX	6	3.2 ± 3.2	80%	none	0%
MOR	5	7.0 ± 2.1†	100%	25.8 ± 23.3‡	100%
M+N	6	4.6 ± 5.0	67%	4.6 ± 6.9	50%

*Mean and standard deviation.

†Significantly different from all other groups, $F(4,26)=2.98$, $p<0.05$; Newman-Kuels, $p<0.05$.

‡Significantly different from Groups NAL, NTX and M+N, $F(4,26)=4.38$, $p<0.01$; Newman-Kuels, $p<0.05$. Groups SAL and MOR did not differ significantly in duration of postictal depression.

minutes after morphine injection and 20 minutes before EBS. Electrophysiological recordings were obtained from animals for up to 60 min following drug administration and EBS. Behavior was scored by two independent observers and in some cases videotaped for later analysis. Evidence of postictal events (i.e., spiking, postictal depression) was obtained from both direct animal observation and examination of EEG records. Only those postictal spikes clearly discernable above baseline EEG (i.e., sharp transients at least twice baseline amplitude) activity were analyzed.

Drugs

Naloxone HCl (Lot No. 80-107) and naltrexone HCl (Lot No. 78-PH-34A) were generously supplied by Endo Laboratories, Inc., Garden City, NY. Morphine sulfate was obtained from Merck & Co., Rahway, NJ. All solutions were made up fresh on the day of injection in sterile 0.9% saline. All injections were given IP and all doses are expressed as the salt. These drug doses were chosen on the basis of earlier pilot work using other doses of naloxone, naltrexone and morphine.

Histological Verification of Electrode Placements

At completion of the experiments all animals were sacrificed with an overdose of sodium pentobarbital and perfused through the heart with 0.9% saline followed by 10% Formalin. Brains were removed, frozen sectioned at 40 microns, mounted on slides, and stained with cresyl violet. The location of electrode tips was verified by reference to the rat brain atlas of König and Klippel [17].

Data Analysis

Only data from animals with the stimulating electrode histologically verified to be within the amygdaloid complex were analyzed. Afterdischarge duration, postictal spiking and postictal depression data were analyzed by analysis of variance (ANOVA). Square root transformations of AD durations were applied to justify use ANOVA procedures [39]. Seizure stage data were analyzed using a Kruskal-Wallis ANOVA [28]. *Post hoc* assessments (i.e., Newman-Keuls, Mann Whitney-U) of individual differences were conducted when appropriate.

RESULTS

Electrode Placement

Data from two Group M+N rats and one Group MOR rat were excluded from further analysis based on electrode tip placements outside the region of the amygdala. Electrode tips for the remaining rats were distributed within the amygdaloid complex over a distance of approximately 1.5 mm in the anterior-posterior plane. No significant correlations between precise electrode tip location within the amygdala and rate of kindling were found.

Seizure Stage and AD Duration

The mean kindled seizure stage for the various groups after drug treatment is shown in Fig. 1. Statistical analysis showed a significant treatment effect, $H(4)=12.69$, $p<0.02$. Subsequent individual comparisons indicated that Groups NAL and NTX showed significantly ($p<0.05$) reduced seizure stages compared to all other groups (i.e., SAL, MOR, M+N).

Mean afterdischarge (AD) durations for experimental groups after drug treatment are shown in Fig. 2. The apparent reduction in AD duration for Groups NAL and NTX failed to reach statistical significance for either ipsilateral, $F(4,26)=2.17$, or contralateral, $F(4,26)=1.74$, recordings [39].

Postictal Depression and Spiking

Some degree of postictal spiking and depression (behavioral and electrographic) were observed in all fully kindled (i.e., Stage 5) animals, although not after every seizure. Opiate effects on postictal events are shown in Table 1.

Compared to the saline injected group (SAL), neither naloxone nor naltrexone, at the doses used in this study, significantly altered the number of spikes observed during the first 60 sec of the postictal period. However, morphine (MOR) significantly ($p<0.05$) increased postictal spiking compared to saline (SAL), naloxone (NAL) or naltrexone (NTX) injected groups. This finding is consistent with previous reports that morphine potentiates postictal spiking in amygdaloid-kindled rats [7,21]. The sequential combination of morphine and naloxone (M+N) produced variable effects on spiking that were not significantly different from saline injected controls (SAL).

A marked reduction in both amplitude and frequency of the postictal EEG was observed in most kindled rats following a Stage 5 seizure as previously reported [25]. This postictal depression of the EEG is accompanied by a behavioral depression in which animals show an almost total cessation of movement. In our studies, recovery from the period of behavioral depression typically begins simultaneously with an abrupt return of the EEG to near normal frequency and amplitude. As shown in Table 1, the mean duration of postictal EEG depression or saline injected rats was 30.0 sec. This was significantly ($p<0.05$) longer than that of naloxone (NAL) or naltrexone (NTX) injected rats. However, both naloxone and naltrexone effectively reduced the behavioral stages of seizure severity. Since postictal depression was not typically seen under less than Stage 5 motoric seizures, it is unclear whether the lack of postictal EEG depression seen in Groups NAL and NTX was due to direct drug effects on postictal events, or only indirectly by way of reduced seizure stage. Morphine did not significantly affect the duration of postictal depression, while naloxone remained effective ($p<0.05$) in reducing the duration of postictal depression when given after a morphine injection (i.e., Group M+N).

EXPERIMENT 2

In Experiment 1, naloxone and naltrexone significantly reduced the behavioral severity of Stage 5 amygdaloid-kindled seizures, but failed to significantly alter afterdischarge (AD) duration. Since a high degree of variability was encountered in AD duration in Experiment 1, the effects of naloxone on amygdaloid-kindled seizures were re-examined in the following experiment using each animal as its own control to reduce inter-animal variability. In addition, a longer pulse duration (i.e., 1.0 msec) than that in Experiment 1 was used, and the criterion for a fully kindled animal was reduced from 3 to 2 consecutive Stage 5 seizures, in order to produce a more rapidly and uniformly kindled animal.

METHOD

Eight adult, male, Long-Evans rats (300–350 g) were im-

TABLE 2
EFFECTS OF NALOXONE (10 mg/kg) ON KINDLED SEIZURES

Rat Number	Treatment Order	SAL AD (sec) IPSI†	SAL AD (sec) CONTRA†	SAL Stage*	NAL AD (sec) IPSI†	NAL AD (sec) CONTRA†	NAL Stage*
29	SAL-NAL	17	3	3	19	0	0
30	SAL-NAL	19	19	5	0	0	0
37	SAL-NAL	90	90	5	7	0	2
39	SAL-NAL	14	0	1	12	0	0
32	NAL-SAL	30	10	3	0	0	0
33	NAL-SAL	25	25	3	48	48	2
34	NAL-SAL	22	22	5	0	0	0
36	NAL-SAL	3	3	3	6	3	0
	Mean=	27.5	21.5	3‡	11.5	6.6	0‡
	S.E.=	± 9.4	±10.3		± 5.7	±5.9	

*Seizure stage defined according to the rating scale of Racine [26].
†IPSI=ipsilateral recording; CONTRA=contralateral recording.
‡Median score.

planted bilaterally with bipolar electrodes aimed for the central amygdala and kindled to a criterion of two consecutive Stage 5 seizures. The procedure was exactly as described in Experiment 1 with the following exception. The EBS parameters in this experiment were 100 Hz, 1 msec pulse duration, biphasic, symmetrical square waves.

After reaching criterion, half of the animals were injected with naloxone (10 mg/kg, IP), and the remaining animals were injected with an equal volume of 0.9% saline. Twenty minutes after injection, the animals were again stimulated and the seizure stage and afterdischarge duration recorded.

After this initial treatment (i.e., naloxone or saline injection), kindling was continued until each animal again reached the criterion of two consecutive Stages 5 seizures. The following day animals originally tested with saline received an injection of naloxone (10 mg/kg, IP), and those initially tested with naloxone were injected with saline. Thus, all animals were tested with both naloxone and saline in counterbalanced order.

Seizure stage data were analyzed using a Friedman two-way ANOVA [28]. Afterdischarge durations at criterion (Time 1) and after (Time 2) naloxone or saline injection (Treatment) were analyzed using a 2 × 2 × 2 (Order × Time × Treatment) repeated measures ANOVA following square root transformation [39].

At completion of the study, animals were sacrificed and electrode tips located histologically as described in Experiment 1.

RESULTS

Electrode Placements

Electrode placements for all animals used in this experiment were histologically verified to be within the boundaries of the amygdaloid region. Placements were similar to those described in Experiment 1. No significant correlations between exact electrode tip placement and rate of kindling were found.

Rate of Kindling

As predicted, the longer pulse duration (i.e., 1.0 msec) used in Experiment 2 resulted in more rapid kindling to Stage 5 (Mean=12.4 days) than that found in Experiment 1 (Mean=19.6 days) using the shorter pulse duration (i.e., 0.1 msec). This observation is consistent with previous reports of maximal kindling with pulse durations in the range of 1.0 msec [13]. The mean AD (±SE) durations at criterion for rats prior to naloxone (NAL) or saline (SAL) injection were 52.4 (±10.6) and 48.0 (±7.6) sec, respectively, for ipsilateral recordings, and 46.1 (±10.5) and 45.7 (±7.4) sec for contralateral recordings.

Naloxone Effects

The effects of naloxone (10 mg/kg, IP) on Stage 5 seizures are shown for individual animals in Table 2.

The average seizure stage following naloxone injection was significantly reduced compared to that seen after saline injection, $\chi^2(1)=8.0, p<0.01$, thus replicating the major finding of Experiment 1. In addition, afterdischarge (AD) durations for both ipsilateral (i.e., stimulated) and contralateral (i.e., recording only) electrodes were also reduced following naloxone injection (10 mg/kg, IP) compared to those observed after saline injection. Planned comparisons indicated that these reductions were statistically significant for both ipsilateral, $t(7)=1.96, p<0.05$, and contralateral, $t(7)=2.12, p<0.05$, recordings. Seizure stage, $\chi^2(1)=3.12, p<0.10$, and AD durations for ipsilateral, $F(1,6)=12.6, p<0.05$, and contralateral, $F(1,6)=28.2, p<0.01$, recordings were also reduced following saline injection, although the reduction was much smaller than that observed following naloxone treatment. This reduced seizure stage following saline injection may be due, in part, to the fact that rats in Experiment 2 kindled to Stage 5 more rapidly than rats in Experiment 1, and therefore underwent fewer seizures in reaching Stage 5. This is important because recent data from our laboratory [31] indicate that earlier stages of kindling are more suscepti-

ble to opiate modification. It is possible that seizures exhibited by rats rapidly kindled to Stage 5, as in Experiment 2, show similar enhanced susceptibility to disruption by opiates, and possibly to the stress of saline injection.

GENERAL DISCUSSION

Results of the present experiments indicate that opiates can modify amygdaloid-kindling in rats. Modification was seen in both ictal and postictal seizure manifestations. The prototypal opiate antagonists, naloxone and naltrexone, reduced both seizure stage severity (Experiments 1 and 2) and afterdischarge (AD) duration (Experiment 2). Morphine increased postictal spiking in fully kindled rats (i.e., Stage 5), but did not significantly affect ictal events. These data support the hypothesis that brain endorphins contribute to the kindling phenomenon.

These results should not be seen as surprising. Epileptogenic properties of morphine have been known for some time [18,38], and several investigators have recently reported that intraventricular injection of morphine sulfate, leucine-enkephalin or methionine-enkephalin produce epileptiform afterdischarges that can be blocked by systemic naloxone injection. Furthermore, kindling develops most rapidly from electrical stimulation of the amygdala; a region extremely high in opiate receptors [19], and enkephalinergic terminals [33]. These finds suggest that possible enkephalin release induced by amygdaloid electrical stimulation may contribute directly to the ictal manifestations of kindled-seizures, and that the development of amygdaloid-kindling may be accompanied by a parallel increase in brain enkephalin levels. This possibility is supported by the recent report that both met-enkephalin and leu-enkephalin levels are increased in rat brain following amygdaloid-kindling [35].

The possible relationship between amygdaloid-kindling and the endorphin system has not been overlooked. For example, Le Gal Le Salle *et al.* [21] recently reported that morphine increased seizure severity and interictal spike frequency in Stage 5 amygdaloid-kindled rats. Frenk *et al.* [7], as well as the present study also found increased interictal spiking induced by morphine, but without concomitant increases in the severity of ictal events. However, failure to observe significant morphine effects on the AD or behavioral components of Stage 5 seizures should be viewed in perspective. Stage 5 may be a relatively insensitive stage at which to observe the effects of morphine on kindled seizure activity. Pinel and Rovner [23] have demonstrated that animals eventually progress to a spontaneous seizure stage following extended kindling. However, AD durations do not markedly increase as kindling is continued on beyond Stage 5, and behavioral changes past Stage 5 require several additional kindling trials. Therefore, at Stage 5 the AD duration may be at or near an apparent upper limit and further increases in seizure severity may be difficult to achieve. As previously suggested by Gaito [11], earlier stages of kindling (e.g., Stage 3) may be more sensitive to the potentiating or anticonvulsant properties of pharmacological agents. Indeed, we [31] have preliminary data indicating that morphine can potentiate amygdaloid-kindling when given at Stage 3.

The present results contrast with previous failures to find naloxone effects on amygdaloid-kindling [7,24]. We have considered several possibilities for the apparent failure of other laboratories to modify amygdaloid-kindling with naloxone. First, each laboratory appears to use a slightly different kindling procedure as well as different stimulation parameters. The procedures and parameters used in the present studies were chosen to produce maximal stimulation of neural elements while avoiding possible tissue injury [3]. They also represent relatively standard kindling procedures. Second, animals in the present study were drug naive (excluding surgical anesthesia during electrode implantation) when treated at Stage 5, in contrast to the procedure of Post *et al.* [24]. Finally, all animals in the present study were kindled to the same seizure stage (i.e., Stage 5) before being tested with naloxone. In the Frenk *et al.* [7] study, previously kindled animals were rekindled prior to drug administration. This procedural difference may be important.

It should be noted that chronic treatment with naloxone has also been reported to facilitate the rate of amygdaloid kindling [5,14]. However, chronic naloxone treatment increases both opiate binding sites [20] and opiate sensitivity [29]. If endorphin release is involved in amygdaloid-kindling as proposed, then it is possible that chronic naloxone treatment may potentiate the rate of amygdaloid-kindling by producing supersensitivity in the endorphin system. However, a single acute dose of naloxone, as used in the present study, would still be expected to produce a functional decrease in endorphin activity along with reduced seizure severity. A further consideration is that in moderate to high doses naloxone can produce epileptiform activity in monkeys [30] and convulsions in mice [6]. The mechanism may involve an interaction of naloxone with the GABA system [6], raising the possibility that modification of kindled seizures by naloxone may involve both brain endorphins and GABA.

A variety of anticonvulsants are known to be particularly effective in blocking both the behavioral and electrographic manifestations of amygdaloid-kindled seizures [1, 2, 36]. It is important to note that we typically see a greater reduction in the behavioral (i.e., motoric) than the electrographic (i.e, AD duration) manifestations of seizure activity by naloxone and naltrexone. This suggests that these agents may be selectively interfering with the recruitment of motor system circuitry during spread of the primary afterdischarge. In this regard, it is interesting to note that opiate antagonists, such as naloxone, have been observed to decrease motor activity in rats [37]. Intra-amygdaloid injections of naloxone also decrease motor activity in rats, while morphine and leucine-enkephalin injections produce an increase (Stone and Berman, unpublished observations). Alternatively, behavioral ratings of seizure severity may be simply more sensitive than AD duration to drug modification. More information is necessary before these questions can be answered.

In summary, we find significant opiate modification of amygdaloid-kindled seizures as predicted by a variety of converging lines of evidence. Clearly, other neural systems also play a role in the development and maintenance of a kindled seizure focus, including the cholinergic, dopaminergic and noradrenergic systems [27]. However, the importance of the endorphins should not be overlooked and future research should be directed toward understanding the interactions of these systems in the regulation of neural activity.

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