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Seizure onset times for rats receiving systemic lithium and pilocarpine Sources of variability

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Abstract

Injection of 30 mg/kg of pilocarpine 24 h after systemic injection of lithium (3 mEq/kg) results in overt limbic motor seizures within about 30 min. Results of several experiments indicated that whereas food deprivation or repeated nociceptive stimulation during the previous 24 h decreased seizure onset times (SOTs) by about 11 to 12 min, food restriction, continuous lighting, or, handling during the previous 7 to 14 days increased SOTs by comparable durations. Early handling before weaning but not injections of clomimpramine also decreased SOTs. A difference of 18 min in the means of SOTs was produced by injecting either 1.0 (increased SOT) or 1.5 mg/kg (decreased SOT) of dexamethasome during the previous 24 h. A strong (multiple r=.87) association between SOTs and the amount of damage within five specific thalamic–limbic nuclei was observed. These results, in conjunction with blood corticosterone levels taken before and after induction of the seizures, suggest the neurochemical mechanisms affecting the range in SOTs could involve the adrenocorticotropic hormone (ACTH)–corticosterone system and influence the amount of post-seizure-induced damage.

Keywords: Lithium/pilocarpine; Seizure onset time; Early handling; Food deprivation; Limbic epilepsy; Brain damage; Dexamethasome; Corticosterone

1. Introduction

A single systemic injection of lithium (Jope and Williams, 1994) chloride (3 mEq/kg) followed 24 h later by a single systemic injection of the central muscarinic agent pilocarpine (30 mg/kg) evokes in male adult rats reliable overt limbic seizures within 1 h. The sustained seizures have been considered an experimental model of status epilepticus (Falter et al., 1992; Hirsch et al., 1992; Honchar et al., 1983; Persinger et al., 1988; Walton et al., 1990). The evolution of the overt behavioral and electrical correlates of the seizure during this brief period (Persinger et al., 1993) resembles the five stages of kindling as defined by Racine (1972). These behaviors include head nods, facial clonus (e.g., ear-twitches), general (whole body) jerks, forelimb clonus, and rearing followed by rapid forelimb clonus and falling. Occasionally, this behavior may be followed by a tonic–clonic seizure.

The subsequent mosaic of quantitative neuronal damage within diencephalic and subcortical telencephalic structures is similar (r=.86) to the quantitative neuronal damage of a single injection of a much larger dosage (380 mg/kg) of pilocarpine only (Turski et al., 1983). About 100 structures, as indicated by Paxinos and Watson (1986), primarily within the thalamus, pyriform (entorhinal) area, and amygdala, exhibit microscopic changes that range from very mild neuronal anomalies to complete neuronal dropout with gliosis (Peredery et al., 2000, Persinger et al., 1988, 1993). The severity of neuronal loss and the patterns of brain damage that emerge during the days to weeks following the onset of the seizures depend upon the postseizure treatment, the duration of the treatment and when it was administered (Santi et al., 2001).

The time *between* the injection of the pilocarpine and the display of rearing and rapid forelimb clonus has been defined as the seizure onset time (SOT). Although this latency is between 20 and 40 min for about 95% of adult male rats, we had observed that group means for SOTs could be quite variable despite constancies in handling and housing. Close observation of behavior and a general understanding of rat physiology suggested that the variations in SOT might be mediated through the more common "stress" pathways such as adrenocorticotropic hormone (ACTH) and corticosterone (Aldenhoff et al., 1983; Falter

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et al., 1992; Joels and de Kloet, 1989; Maccari et al., 1992; Missaghi et al., 1992). Our working hypothesis has been that this pathway, beginning with the release of corticotropin releasing factor (CRF), is a major (but not only) determinant of SOTs.

During the last 10 years we have conducted experiments to discern the types of stimuli, presented before weaning, within 2 weeks before the injection of lithium, during the 24 h period between the lithium and pilocarpine injections and immediately after the injection of the pilocarpine that might affect SOTs. We have employed procedures that directly or indirectly affect the ACTH–corticosterone response. These procedures have ranged from daily handling during infancy to injection of synthetic steroids.

Variables or factors that affect SOT, even by a few minutes, may be important. In unpublished studies we found moderately positive (r=.50) correlations between the amount of neuronal loss within the areas of the endopiriform nuclei, corticoamygdaloid nuclei, lateroposterior nuclear group of the thalamus and the nucleus reuniens of the thalamus and SOTs. Differences in SOTs of only about 5 min were associated with greater severity of neuronal dropout within specific structures. These results indicated that a more precise understanding of the variables that affect SOTs might ultimately reveal the mechanisms that affect the patterns and the severities of brain damage that follow the induction of these seizures.

2. Method

2.1. Subjects

A total of 319, male Wistar albino rats (Charles River, Quebec) were employed as subjects in a total of 13 different experiments. All rats were between the ages of 80 and 100 days.

2.2. Procedure

2.2.1. General

Unless specified otherwise, all rats were housed in groups of three (of the same sex) in standard wire cages within temperature controlled rooms. The L/D was 12:12 with light onset at 0700 h local time; precipitation of the seizures always occurred between 1000 h and 1700 h. After at least 2 weeks of habituation to the colony conditions, the seizures were evoked by injecting subcutaneously (sc) 3 mEq/kg (3 mEq/cc) of lithium chloride into the left flank. Twenty-four hours later, the overt seizures were precipitated by a single subcutaneous injection of 30 mg/kg of pilocarpine (30 mg/cc).

SOT was directly observed by placing each rat in a glass aquarium (always in the same room, separate from the treatment or maintenance area). A total of six to eight rats were seized during any given session and there was no more than one session per day. The latency for forelimb clonus, rearing and falling (the criterion response) was recorded to the nearest second by a stopwatch. All analyses were completed by SPSS software using a VAX 4000 computer. η values, which are equivalent to correlation coefficients, were extracted as additional indicators of the strengths of the statistically significant treatment effects. All experiments were completed over a 10-year period between 1991 and 2000. The protocols had been approved by the local Animal Care Committee.

Most of the rats employed in these studies were not terminated after the induction of the seizures but were injected (subcutaneously) immediately after the onset of the seizures or within 1 h after the injection of the pilocarpine with between 25 to 30 mg/kg of acepromazine (Persinger et al., 1993) or 100 mg/kg of ketamine (Santi et al., 2001) in order to promote survival. These rats were employed in more than 20 undergraduate and graduate theses involved with histomorphology or behavioral sequelae.

Verification of the validity of the behaviors antecedent to and defining the SOTs was completed by implanting electrodes within the limbic system and recording the EEG following the injection of pilocarpine. Sample rats were injected with sodium pentobarbital (65 mg/kg ip) and positioned in a stereotaxic frame. They received Teflon-coated electrodes (124 μm in diameter) implanted bilaterally at the level of the stratum radiatum in the CA1 portion of the hippocampus. One week later and after baseline recording, EEG recordings were obtained.

As can be seen in Fig. 1, the behavioral sequences associated with head movements (B) were associated with periodic spiking, quite discernable from baseline (A). Tremor and facial clonus (C) that emerge about 2 to 10 min after the injection of the pilocarpine were associated with higher amplitude spiking. The behaviors that defined SOTs, forelimb clonus and rearing (D) were associated with clear paroxysmal discharges. The persistence of the seizures (status epilepticus) are evident in part E. If adult male rats were not injected with acepromazine, ketamine or a related compound mortality exceeded 90% within 72 h (Santi et al., 2001). The relationships between the areas in which damage was measured microscopically and different types of seizures, recorded electroencephalographically, by other researchers have been reported elsewhere (Peredery et al., 2000).

2.2.2. Experiment I

Twelve rats were deprived of food for 24 h (beginning immediately after the lithium injection) while another 12 rats served as the ad libitum reference group. SOTs were precipitated by the pilocarpine.

2.2.3. Experiment II

In four separate blocks, 36 rats were injected with lithium and then assigned to one of two groups (18 each). One group remained in the home cages for 4 h. Each rat from the other group was placed upon a standard hot plate (55°C) until he either lifted and licked the hind paw twice or 60 s had elapsed.

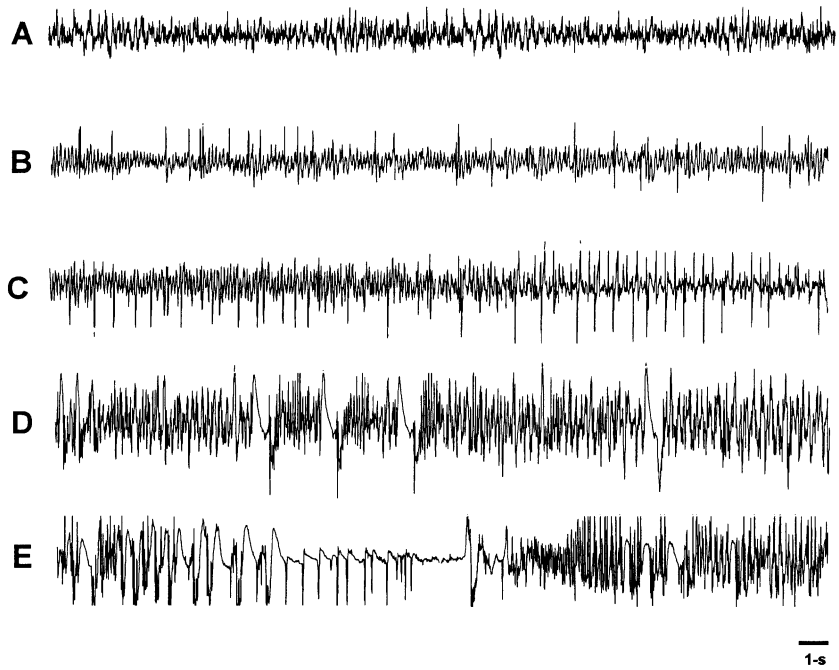

Fig. 1. Electrical recordings from the hippocampus associated with different behaviors before and following injection of pilocarpine. (A) Baseline (predrug), (B) head movements and tremor, (C) facial clonus, (D) forelimb clonus and rearing, and (E) about 30 min after onset of status epilepticus following (D).

The rat was then returned to the home cages. This procedure was repeated every hour, four times, for each rat. At the end of the 4 h, all rats were injected with pilocarpine.

2.2.4. Experiment III

After injection of the pilocarpine, 24 rats were assigned to one of four treatments (6 per treatment): (1) successive 10 s on/10 s off intervals of 80 dB 10,000 Hz tones, (2) whole body rotation at 33 RPM: the rat was placed in a plastic cage attached to a modified phonograph turntable, (3) exposure to cedar wood oil that was placed in wood shavings under the observation cage, or (4) home cage condition (undisturbed controls). The treatments were continuous until the seizures occurred and SOTs were recorded.

2.2.5. Experiment IV

Twenty-four rats were injected with lithium and then injected (other flank) with either (six rats/treatment): isotonic saline, or 0.4 mg/kg or 1.0 mg/kg or 1.5 mg/kg of dexamethasome (DXM); 17 h later (7 h before pilocarpine and 3 h before the first hot plate exposure), they were injected again with the same dosage of DXM. The dosages were selected to simulate the conditions of Kant et al. (1988). Each rat was then placed on the hot plate (as in Experiment II) once every hour, four times. Pilocarpine was then injected immediately after the fourth hot plate exposure and SOTs were recorded.

2.2.6. Experiment V

Prednisolone (Sigma) was dissolved in tap water (1 mM) and given ad libitum in place of the drinking water for 2 weeks to nine rats; another nine rats of the same age consumed tap water only. The dosage and duration of treatment with this exogenous glucocorticoid were selected because they have been shown by Eckland et al. (1991) to exert a profound suppression of CRF mRNA within the parvocellular compartment of the paraventricular nucleus of the hypothalamus.

2.2.7. Experiment VI

Fourteen rats were housed individually in standard Gerbrand running wheel cages for 5 days during which time food and water were available ad libitum. Then, for three successive days, they were allowed access to food and water for only 1 h/day. Details for this procedure, the activity–stress paradigm, have been described by Kant et al. (1988). The numbers of wheel rotations per day were recorded. Another 10 rats served as the single-housed, reference group, and were given a similar food-deprivation schedule. On the fifth day after the beginning of the procedure (food availability for 1 h/day), pilocarpine was injected and SOTs were recorded.

2.2.8. Experiment VII

In Block A of the experiment (split-litter design), four litters were culled to eight pups each. Half of the pups in each litter were injected subcutaneously with the antidepressant clomimpramine (1 mg/kg; twice per day) between postnatal days 4 and 21. This procedure has been reported to produce a suppression of REM sleep and to generate behaviors in adult rats that share many of the characteristics of human endogenous depression (Stout and Nemefoor,

1994; Vogel et al., 1990). The other pups in each litter were injected with 0.9% saline. The color code, applied with a nontoxic magic marker (Persinger, 1977), was counterbalanced for the two treatments between litters. The rationale for this treatment was derived from the significant overlap of symptoms and biochemistry, particularly elevated cortisol and ACTH (Stout and Nemefoor, 1994) that is found in patients who have been diagnosed with either partial complex (limbic or temporal lobe) epilepsy or depression.

In Block B of the experiment eight litters were culled to eight pups each. Between postnatal days 2 and 21, each pup from four of the litters was handled daily for 2 min (each). After the handling the rat was placed in a separate box, containing the usual corncob bedding; the rats were returned to their mothers only after the daily handling for a given litter had been completed, i.e., about 20 min. The pups in the other litters were never handled except when the cages were changed on postnatal days 15 and 18. After weaning, rats of the same sex were housed three per cage.

Two rats that had received the antidepressant and two of their litter mate controls were selected from each of the four different litters ($n=8$ treated; $n=8$ saline-injected) and two rats from the handled litters and two from the non-handled litters ($n=8$ handled; $n=8$ not handled) were tested when they were approximately 100 days of age. They were injected with the standard lithium dosage and then 24 h later with the standard dosage of pilocarpine; SOTs were recorded.

2.2.9. Experiment VIII

Previous work (Persinger et al., 1988, 1993) had demonstrated that a 24 h interval between the lithium and pilocarpine injection was not essential for the display of limbic seizures. Estimates from this work suggested a delay of about 4 h evoked similar SOTs. To insure this consistency, a total of 11 rats were injected with lithium 24 h before the injection of the pilocarpine and another 11 rats were injected 4 h before the injection of the pilocarpine. SOTs were recorded.

2.2.10. Experiment IX

Eighteen rats served as subjects. Nine of the rats (three cages) were removed from their home cages and handled singly immediately adjacent to the cage within the maintenance room for 2 min on 10 consecutive days. The other nine rats (three cages) served as the undisturbed, non-handled reference group. The cages were on the same side of the rack as those of the handled rats so they would be exposed to the same sounds and motions. The rats were injected with lithium and then 4 h later with only 20 mg/kg of pilocarpine. The reduced dosage of pilocarpine and latency between the lithium and the pilocarpine (Persinger et al., 1988) was employed so that a larger potential range in SOTs might be obtained.

In previous studies (Persinger et al., 1993), the coefficient of variation (standard deviation divided by the mean, quantity multiplied by 100) did not differ significantly between 30 and 20 mg/kg of pilocarpine. To insure that the previous pattern of results was still valid, an additional 22 rats were recruited in this experiment. All rats received the same, usual treatment of lithium and pilocarpine except the interval between the injections was 4 h for one group ($n=11$) and 24 h for the other ($n=11$).

2.2.11. Experiment X

Within 30 min after injection of the lithium (23.5 h before the injection of the pilocarpine) 16 rats were restrained for approximately 5 min (range: 2 to 9 min). During this period blood was removed by a small gauge needle (100 μl) from the tail vein after it had been warmed by submersion for 30 s in a water bath. The rats were placed in the observation cage and SOTs were recorded. Five days later, a second blood sample was collected.

The corticosterone levels were determined by a commercial assay kit. The correlation between SOT, relative change in body weight ((Day 5 weight − baseline weight)/baseline weight) over the 5 days between the induction of the seizure and the second blood measure and the corticosterone levels were calculated. An additional eight rats, one from each of the cages from which the rats had been taken for the blood sampling, served as controls.

2.2.12. Experiment XI

SOTs in one group ($n=22$) were observed while the rats were ambulatory within the observation cages (the typical paradigm). A second group ($n=29$) had been restrained in commercial, multiribbed restrainers within 5 min after the injection of the pilocarpine until the onset of the overt seizures. In the latter group, SOT was determined by the pattern of muscle movement within the restrainer. Approximately six to eight rats, each assigned to one of the two treatments, were tested for seven sequential days.

2.2.13. Experiment XII

Nineteen rats were exposed to continuous light instead of the L/D 12:12 cycle for 7 days. Another 23 rats, housed in a separate room, were exposed to the normal light schedule. SOTs were measured.

2.2.14. Experiment XIII

To discern if the temporal distributions of SOTs were correlated with the proportions of the subsequent neuronal loss within specific nuclei, the scores for damage in each of approximately 100 structures (Paxinos and Watson, 1986) and SOTs were obtained for 33 male rats that had been seized but had not received any of the manipulations in this study. These rats were randomly selected (three to four per experiment) from the reference or control groups from Experiments I through XII. The rats had received the standard postseizure treatments of acepromazine shortly after the display of rapid forelimb clonus and rat chow mush during the following week or until they returned to

eating hard food (Persinger et al., 1988, 1993). The rats had been killed by decapitation 30 days after seizure inductions.

The brains were fixed in ethanol–formalin–acetic acid (EFA) because it reveals excellent, clearly discriminable morphology in neuronal soma and glial nuclei when stained with toluidine blue O (Persinger, 1977). Each brain was sectioned at 10 μm by a microtome between the posterior and anterior commissures. Only those regions have demonstrated conspicuous damage (Persinger et al., 1993).

The presence of damage within a given structure was defined by neuronal loss, gliosis or other anomalies at 100× and 400× in each of the 210 (although only about 100 display discriminable damage with this treatment) structures designated by Paxinos and Watson (1986). The ratings for each structure, which reflected the extent of damage, ranged from 0 (*no damage*) to 10 (*maximum damage*), had been completed by trained histologists who were not aware of the SOTs or the conditions of the animals.

The damage score for each structure was calculated by observing all of the sections for a particular structure and allocating a score of either 0 or 1 for damage (Persinger et al., 1994). The damage score for each structure was the proportion of slides (between four and eight slides per structure) with a score of 1. For example if five of five sections received a score of 1, the score for the structure was 10. If four of the eight sections showed scores of 1 and four showed scores of 0, the score for the structure was 5. Scores for this method of estimating neuronal loss within each structure have reliably demonstrated strong bivariate correlations ($r > .80$) with rats' quantitative scores for complex behaviors such as conditioned taste aversion (Persinger et al., 1994) and spatial memory (Persinger et al., 1994; Santi et al., 2001).

Step-wise multiple regression analysis was completed with the SOTs as the dependent variable and the values of neuronal damage for each of the 100 structures as the independent variables. Because there were only 33 rats, the numbers of variables allowed to enter the equation were restricted to five. Plots were extracted to insure intrinsic linearity of the relationship between the proportion of damage and the SOTs for the partial correlations. The means and standard deviations (S.D.s) for the proportions of damage for each of the structures were compared to those of previous studies.

3. Results

A brief summary of the results of the experiments is shown in Table 1. The differences in minutes between the mean SOTs for the groups receiving the treatments and their respective control or reference groups are indicated. As

Table 1
Mean deviations for SOT (in minutes) for groups of rats given various treatments compared to their control or reference groups

Experiment number	Procedure	SOT difference (in minutes)	% change
Immediate treatments			
III	Postpilocarpine stimulation		
	(a) sound	+3.5	11
	(b) vestibular	+5.0	16
	(c) smell	−0.3	1
XI	Postpilocarpine restraint	−0.1	1
Treatment during previous 24 h			
I	24-h food deprivation	−12.1	33*
II	4× hotplate exposures	−11.1	30*
IV	DXM		
	(a) 0.4 mg/kg	−2.0	7
	(b) 1.0 mg/kg	+9.0	30**
	(c) 1.5 mg/kg	−9.0	30*
X	Blood sample	−3.1	11
VIII	Lithium–pilocarpine interval:		
	4 vs. 24 h	−5.9	17
Treatment during previous 10 to 14 days			
V	Oral prednisolone	+5.8	16
VI	Food restriction + running wheel	+6.2	31*
IX	Daily, brief handling	+11.0	40**
XII	Continuous lighting	+12.0	36*
Early treatment (preweaning)			
VII	Norepinephrine depletion	−5.0	12
	Daily handling	−10.4	46*

The percentages for the relative increases or decreases in SOTs following the treatments are also shown.

* Indicates statistically significant $P < .01$ as determined by one-way analysis of variance.

** The means were not significantly different but there was significant ($P < .01$) heterogeneity of variance between treatments.

additional information the percent increases or decreases of the SOTs following the treatments compared to the control groups are included. Only treatment effects at $P < .01$ were accepted as statistically significant.

3.1. Experiment I

The rats that had been deprived of food for 24 h displayed significantly [$F(1,22) = 11.45$, $P < .01$; $\eta = .59$] faster [24.1 (8.5) min] SOTs than did the ad libitum reference group [36.2 (9.0) min]. There was no significant ($F < 1$) difference between mean body weights for the two groups.

3.2. Experiment II

Rats that had been exposed to the hot plate once per hour for four trials before the pilocarpine injection displayed significantly [$F(1,34) = 11.52$, $P < .01$; $\eta = .50$] faster SOTs [26.1 (8.2) min] than rats from the reference group [37.2 (11.2) min]. Again, there were no statistically significant differences in body weights between groups.

Repeated measures analysis of variance for the foot lick latencies across the four trials was significant [$F(3,51) = 3.29$, $P < .05$]. The means and S.D.s for these latencies for the first, second, third and fourth hour were: 41.5 (13.8), 34.3 (14.5), 32.6 (16.6), and 40.9 (16.6) s, respectively. Post hoc *t* tests indicated the major source of the difference was between the first and third hour. SOTs were significantly correlated ($r = .51$, $P < .05$) with the foot lick latency for the third hour only.

3.3. Experiment III

Analysis of variance demonstrated no statistically significant differences [$F(3,20) = 0.96$, $P > .05$] in SOTs between the four treatments that were introduced immediately after the injection of the pilocarpine. The means and S.D.s for the four treatments were: sound: 33.5 (7.7) min, body rotation: 35.0 (11.2) min, olfaction: 29.7 (10.7) min and home cage reference: 30.1 (12.3) min. There were no significant differences in body weights between groups.

3.4. Experiment IV

Analysis of variance demonstrated significant treatment effects [$F(3,20) = 12.26$, $P < .001$; $\eta = .60$]. The means and S.D.s for SOTs for the groups were: saline injected: 30.1 (4.2) min, 0.4 mg/kg DXM: 28.0 (4.0) min, 1.0 mg/kg DXM: 39.1 (11.2) min and 1.5 mg/kg DXM: 21.1 (5.2) min. Post hoc analysis (Tukey, $P < .05$) indicated that the primary source of the difference was between the group that received the 1.5 mg/kg of DXM relative to the groups that received either the saline or the 0.4 mg/kg of DXM that did not differ significantly from each other.

Analysis of variance also indicated significant group differences for the latency of foot licking after placement upon the hot plate [$F(1,53) = 7.93$, $P < .01$]. The means and S.D.s for the above groups were: 37 (9), 46 (14), 38 (10) and 57 (3) s, respectively. Post hoc analysis indicated that the 0.4 and 1.5 mg/kg DXM groups displayed slower licking latencies compared to controls or the 1.0 mg/kg DXM groups.

3.5. Experiment V

There was no statistically significant difference [$F(1,16) = 1.46$, $P > .05$] between SOTs for the rats that had consumed prednisolone in the water supply for 2 weeks [40.9 (12.8) min] and the tap water controls [35.0 (7.0) min]. However, the rats that consumed the prednisolone at this dosage weighed significantly [$F = 11.56$, $P < .01$; $\eta = .65$] less than did the controls. The means and S.D.s were 405 (41) and 462 (28) g, respectively.

3.6. Experiment VI

Rats that had been exposed suddenly to the food-restricted diet with options for running wheel activity displayed significantly [$F(1,22) = 8.48$, $P < .01$; $\eta = .53$] slower SOTs [26.2 (6.5) min] than food-restricted controls [20.0 (2.0) min]. The rats in the running wheel group lost 19% (8%) of the baseline body weight compared to the 8% (4%) weight loss that was recorded for the reference group [$F(1,22) = 10.26$, $P < .001$; $\eta = .57$]. SOT was negatively correlated ($r = -.55$, $P < .05$) with the percentage of weight loss for the experimental (the running wheel) group only.

3.7. Experiment VII

There was no significant [$F(1,14) = 0.61$, $P > .05$] difference in SOT between rats that had received the daily, preweaning injections of the norepinephrine-depleting treatment [34.2 (8.3) min] compared to rats that had received saline injections [39.3 (16.4) min]. There were also no significant ($F < 1$) differences in body weights between the two groups (grand mean and S.D. = 381 and 45 g, respectively).

However, the rats that had been handled daily during their preweaning development displayed significantly [$F(1,15) = 8.40$, $P < .01$; $\eta = .67$) faster SOTs [25.9 (2.6) min] relative to the nonhandled reference group [36.3 (8.4) min]. There was no significant difference between the body weights of the group that had been handled or not handled before weaning (grand mean = 402 g, S.D. = 36 g).

3.8. Experiment VIII

Analysis of variance indicated no statistically significant difference [$F(1,20) = 3.72$, $P = .06$; $\eta = .41$] between the SOTs for the group that received the pilocarpine 24 h after the injection of the lithium [32.4 (7.6 min)] or 4 h after the injection of the lithium [26.5 (5.8) min].

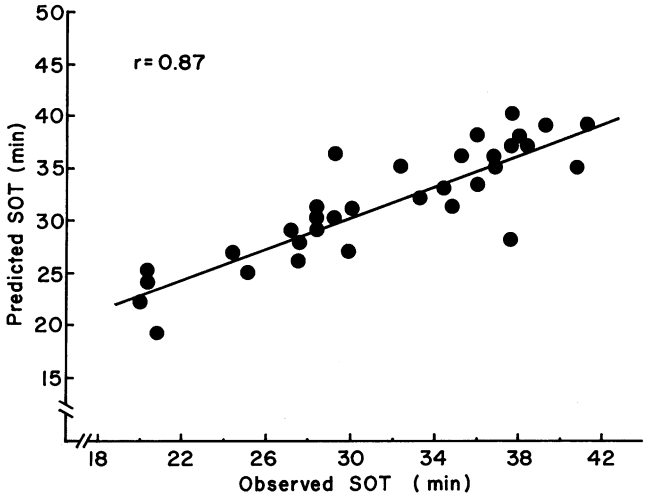

Fig. 2. Scattergram between the observed SOTs and predicted SOTS for rats based upon the amount of damage within five nuclei within the thalamus, hippocampus, and amygdala measured 30 days after induction of the seizures.

3.9. Experiment IX

Rats that had been handled daily for 10 days exhibited SOTS [38 (15) min] that were not different [$F(1,16) = 0.50$, $P > .05$] from the nonhandled reference group [27 (3 min) min]; however Bartlett's Box for heterogeneity of variance was significant ($F = 14.91$, $P < .001$) and was due to the greater variability in SOTs within the group that had been handled daily for 10 days. A nonparametric (Kruskal–Wallis) test also failed to indicate any group difference ($\chi^2 = 0.67$, $P > .05$) in SOTs.

3.10. Experiment X

There were no statistically significant differences between the SOTs for the rats that had been restrained for about 5 min for the blood sample 23.5 h before the pilocarpine injection and nonhandled cage mates [$F(1,23) = 1.69$, $P > .05$]. The means and S.D.s for the two groups were 27.2 (5.8) and 30.3 (6.3) min, respectively.

The means and S.D.s for the corticosterone levels for the baseline period (about 30 min after injection of lithium and about 5 min of restraint) and 5 days after the induction of the seizures and postseizure treatment (e.g., acepromazine, exposure to food mush) were 9.3 (7.8) and 15.9 (9.4) μg/dl. The corticosterone levels 5 days after the seizure were significantly higher [paired t test $(15) = 3.08$, $P < .05$] than for the baseline period. There was no significant correlation ($\rho = .02$) between SOTs and the antecedent level of corticosterone. However, the SOTs were significantly and inversely correlated ($\rho = -.52$, $P < .05$) with the corticosterone levels 5 days later.

Rats that displayed the longest SOTs lost the least amount of relative body weight during the subsequent 5 days ($\rho = .46$, $P < .05$) and the magnitudes of the corticosterone levels 5 days later were lowest for rats that had lost relatively less weight ($\rho = .70$, $P < .001$). The mean and S.D. for the percentage of weight change for the 5 days for the group was 2.5% (11.1%).

3.11. Experiment XI

The SOTs for rats that had been physically restrained within a few minutes after the injection of the pilocarpine did not differ significantly from the rats who were ambulatory before the onset of the overt seizures [$F(1,49) < 1.0$; $P > .05$]. The means and S.D.s for the SOTs were 29.5 (7.5) and 29.3 (6.3) min, respectively.

3.12. Experiment XII

The rats that had been exposed for 7 days to continuous colony lighting displayed significantly slower [$F(1,40) = 18.62$, $P < .001$; $\eta = .57$) SOTs than did matched controls that had been exposed to the normal schedule of L/D 12:12. The means and S.D.s for the two groups were 46.8 (11.6) and 34.4 (6.7) min, respectively. Although the group exposed to the continuous lighting displayed greater heterogeneity in the SOTs, a Kruskal–Wallis nonparametric analyses verified the statistically significant difference between the two groups ($\chi^2 = 12.69$, $P < .001$).

3.13. Experiment XIII

The results of the multiple regression analyses indicated that linear combinations of the amount of damage within five structures resulted in a multiple correlation of .87 [$F(5,27) = 17.12$, $P < .001$; adjusted $r^2 = 72\%$]. A scatter-

Table 2
Names of structures and their means and S.D.s for ranks of damage (possible range 0 through 10), partial slopes (B), standard errors of B (SEB), multiple-r (MR) values and changes in r^2 (amount of variance explained) with the inclusion of each successive structure that was associated with SOTs

Variable	Mean	S.D.	B	SEB	MR	Change in r^2
(1) Gelatinosus thalamic nucleus	3.5	4.1	0.55	0.15	0.58	32%
(2) Parasubiculum	3.3	3.6	−0.62	0.17	0.73	19%
(3) Corticoamygdaloid nucleus (posterolateral)	6.4	3.0	1.11	0.24	0.79	6%
(4) Intermediodorsal thalamic nucleus	3.5	3.4	0.65	0.19	0.85	10%
(5) Basomedial (posterior) amygdala nucleus	5.9	4.3	−0.28	0.14	0.87	5%
Constant			24.0	2.2		

gram between the predicted SOTs from the proportion of damage within these structures and the observed SOTs is shown in Fig. 2. The standard error of the estimate for the regression line was 3.3 min.

Table 2 shows the variables that entered the equation, the means and S.D.s for the damage, the partial slopes (partial regression coefficients), the standard errors for the slopes, and the changes in r^2. All variables that entered the equation were statistically significant ($P < .01$). The equation indicated that longer SOTs were associated with more damage within the gelatinosus nucleus of the thalamus, posterolateral corticomedial amygdaloid nucleus, and interanteriomedial nucleus of the thalamus but proportionally less damage within the parasubiculum and anterior part of the basomedial nucleus of the amygdala.

Internal reliability for the equation's structure was discerned by completing analyses with the first 17 and the last 16 cases, separately. The first four variables entered (although in different order) with the same directions of slopes for the equations generated for the two sets of cases. The structures that entered the equation were not those most damaged; they are listed in Table 3. In addition, sample structures with comparable amounts of damage to those that entered the equation are also shown for comparison in Table 4.

Nonparametric correlations (ρ) showed significant ($P < .01$) associations between the SOTs and the proportions of damage within the following structures (ρ values in parentheses): parasubiculum (− .49), piriform cortex (.36), dorsal endopiriform nucleus (.38), ventral endopiriform nucleus (.38), posteromedial cortical amygdaloid nucleus (.53), cortex–amygdala transition zone (.36), basomedial amygdaloid nucleus (.38), medial amygdaloid nucleus, anterodorsal part (− .47), marginal zone of the medial geniculate (− .43), suprafascicular thalamic nucleus, parvocellular part (.36), reuniens thalamic nucleus (.38), ventral reuniens thalamic nucleus (.40), gelatinosus thalamic nucleus (.62), and the islands of Calleja (− .36).

Table 3
Means and S.D.s for the proportion of damage (range 0 to 10) for the most damaged structures

Structure	Mean	S.D.
Reuniens nucleus (ventral)	9.6	1.4
Piriform cortex	9.4	1.2
Substantia nigra (reticulata)	9.4	1.8
CA1 hippocampus	9.2	1.1
Endopiriform nucleus ventral	9.2	1.3
Posteromedial cortical amygdaloid nucleus	8.9	1.8
CA2 hippocampus	8.6	2.3
Laterodorsal thalamic nucleus (ventrolateral)	8.5	2.2
Laterodorsal thalamic nucleus (dorsomedial)	8.5	2.5
Posterior thalamic nuclear group	8.4	2.7
Basolateral amygdaloid nucleus (ventral)	8.2	3.3
Endopirifrom nucleus (dorsal)	8.2	2.7
Anteriomedial thalamic nucleus	7.9	3.7
Mediodorsal thalamic nucleus (medial)	7.9	3.7
Lateroposterior thalamic nucleus (laterorostral)	7.6	3.2
Lateral amygdaloid nucleus (ventrolateral)	7.6	3.7
CA3 hippocampus	7.5	2.9
Insular cortex (agranular)	7.1	3.5
Mediodorsal thalamic nucleus (lateral)	7.0	3.2

Table 4
Examples of other structures that were damaged within the range of (3.5 to 6.4) the structures that entered the equation

Structure	Mean	S.D.
Entorhinal cortex	4.3	2.7
CA4 Hippocampus	4.7	3.6
Subiculum	5.1	3.6
Cingulate gyrus (area 2)	4.5	4.2
Claustrum	5.9	3.7
Caudate–putamen	4.0	2.1
Basomedial amygdaloid nucleus	3.9	4.9
Lateral amygdaloid nucleus	3.6	4.7
Lateroposterior thalamic nucleus (medialrostral)	6.0	3.5
Centrolateral thalamic nucleus	3.8	3.5
Paratenial thalamic nucleus	5.2	4.5

4. Discussion

During the last 10 years we have been identifying the behavioral variables that affect the SOTs for rats injected subcutaneously with lithium and pilocarpine. Although an acceleration or inhibition of SOTs by only 10 min for groups exposed to different behavioral treatments might be considered minor for the behaving organism, this duration involves substantial neuronal activity. Assuming 100 ms for functional changes within the synapse correlated with patterns of action potentials or the sequestering of ligands, a difference of 10 min could indicate the existence of neuronal conditions that might impede or facilitate the spread of the electrical seizures by almost four orders of magnitude.

The results of our present studies, summarized in Table 1, indicated that stimuli presented after the injection of the pilocarpine did not significantly affect the SOTs. Loud sounds, whole body rotation, strong olfactory stimuli, and even physical restraint (Seltzer et al., 1986) did not significantly affect the SOTs compared to control groups. Considering the changes in brain and blood chemistry during the 30 min following the injection of pilocarpine (Honchar et al., 1983), one would not expect a priori that the chemical correlates of these behavioral procedures could compete with the cataclysmic changes elicited by this powerful muscarinic agent.

However, the behavioral treatments during the previous 24 h reduced the SOTs by about 10 min or to about one-third of control values. Food deprivation during the previous 24 h and four separate exposures to a hot plate produced a comparable decrease in SOT. Briefer, presumably nociceptive, stimulations such as exposing the tail to warm water and extraction of blood about 23 h before the injection of the pilocarpine did not significantly affect the SOTs.

In other unpublished studies designed to discern if exposures to 0.5 Hz rotating magnetic fields within the 1 to 10 mT range 24 h before the injection of pilocarpine affected SOTs, we had found no significant difference between the experimental and sham-field exposed groups. These rats had been removed from their home cages and placed in the new, experimental or sham-field settings. Reexamination of the data indicated that the SOTs of both groups were about 12 min faster than their contemporary control groups that had not been disturbed in their home cages during the previous 24 h.

Treatments whose durations were longer than 24 h and involved the previous 1 to 2 weeks produced the opposite effect upon SOTs. The restriction of food plus opportunity to access running wheels as well as exposure to continuous lighting resulted in delays of SOT by about 10 min. These values were about one-third longer than rats exposed to normal conditions. The daily, brief handling of rats also delayed the SOTs by a comparable time. However, the differences were not significant because of the heterogeneity of variance in the handled group and may suggest that not all rats responded similarly to the different handlers.

There has been a long history in neurobiology and psychobiology of the effects of early experience upon adult behavior (Haltmeyer et al., 1967; Meaney et al., 1994). We were surprised that the daily, preweaning injections with a compound known to enhance depletion of norepinephrine and produce ''depressive'' behaviors in adult rats (Vogel et al., 1990) did not affect SOTs significantly. Daily preweaning handling, which also affects the numbers of mast cells within the thalamus of postweaned rat pups (Persinger, 1977), reduced the SOTs by about one-half the time compared to nonhandled controls.

The significant role of early experience upon the subsequent vulnerability of the mammalian brain to the onset of electrical seizures and their overt correlates has been suggested for complex partial seizures in humans (Morton et al., 1990; Snead, 1989; Stout and Nemefoor, 1994). However, evidence based upon experimental results are obviously absent. The results of the effects of preweaning manipulations indicate that the largest changes in adult SOTs occurred without, presumably, traumatic damage to neurons during development.

Daily handling is more likely to have affected the microorganizations within the developing brain and how it responded to the lithium/pilocarpine-invoked changes in brain chemistry. The effects of repeated tactile stimulation may be more potent than suspected. Baker and Persinger (1995) showed that, for female rats, daily stimulation of the vagina shortly after opening significantly decreased the SOTs compared to handled-only controls when these rats were seized as adults. Our interpretation of the results are consistent with but not exclusively proof of the role of the ACTH–corticosterone system in the latency of SOTs. Rats that received 1 mg/kg of DXM during the previous 24-h period displayed longer SOTs while those that received 1.5 mg/kg exhibited shorter SOTs. The difference between the means for the two groups was about 18 min. These results suggest that almost the complete range in differences in SOTs produced by behavioral manipulations occurred within a relatively small window of blood levels of this synthetic steroid.

If the behavioral manipulations induced similar changes within the brain, then the acute procedures during the previous 24 h such as food deprivation and several exposures to nociceptive (hot plate) stimuli would have been associated with the highest levels of ACTH–corticosterone levels and hence the fastest SOTs. Maintained exposure to behavioral manipulations over 1 to 2 weeks, such as a continuous light regime or food restriction and an option to run would have been associated with a less intense but maintained level of circulating steroids as the organism adjusted to the schedule. These rats exhibited longer SOTs.

The application of this explanation to the reduced latencies for SOTs in rats that were handled before weaning would require an elevated corticosterone response. Levine (1962) and Haltmeyer et al. (1967) reported that rats handled throughout the preweaning period showed a more rapid and sustained steroid response to nociceptive stimuli, such as electric shock, compared to nonhandled rats. These changes occurred within about 10 min. If we assume that the handling and subcutaneous injection of lithium 24 h previously and the placement of the animals in the (novel) observation cage after the subcutaneous injection of pilocarpine constituted nociceptive stimuli, then the reduction in SOTs compared to the controls might be rationalized.

In addition to isolating the potential sources for the range in SOTs, these experiments were important because they showed that SOTs were strongly related to the amount of subsequent neuronal loss within specific structures within the brain. The proportions of damage found within the thalamic gelatinosus nucleus, interomedial part of the medial dorsal nucleus of the thalamus and the posterolateral corticomedial nucleus of the amygdala, about 30 days after the induction of the seizures, increased in rats from control groups as a function of their SOTs.

There are several potential interpretations of this pattern of damage associated with almost three-quarters of the variance in SOTs. Our explanation is that individual differences in microstructural organization, receptor expression, or distributions of cholinesterase activity within these nuclei control the exact timing of the collapse of the GABA system that allows the motor expression of the seizures. When the collapse occurred, the resulting excitation produced greater cell loss than if the collapse would have occurred more quickly.

The internal reliability of the relationship between the damage in these structures and the SOTs was indicated by the similarity of the first four structures that entered when half of the population was analyzed. The specificity of the structures rather than the amount of damage, per se, was shown by the numerous structures displaying

more damage or a greater range of damage that did not enter the equations.

Because at least 30 days had elapsed between the inductions of the seizures and the histomorphological examinations, we cannot identify the many processes during this period that may have affected our results. We appreciate that losses of portions of a neuronal population within a functional nucleus during the 30 days between the induction of the seizure and the removal of the brains could have been due to excitotoxic death, apoptotic death or transneuronal degeneration from insufficient stimulation. All rats were treated similarly during the recovery period. Because we have found in unpublished studies that incidental postseizure exposure to fire alarms enhanced neuronal loss within the suprageniculate nucleus of the thalamus and tactile/nociceptive stimulation of the tail during blood extractions enhanced neuronal loss within the posterior nucleus within the ventral tier of the thalamus, such homogeneity of postseizure procedures is important.

The results of the present study support this suggestion. The strongest bivariate association occurred between SOTs and the amount of damage within the gelatinosus nucleus of the thalamus. This structure, which has been inferred to contain copious numbers of cholinergic receptors on the basis of its staining density for acetylcholine esterase, receives input from the caudal subnucleus of the spinal trigeminal nucleus (Faul et al., 1985). Rats that displayed the longest latency to display the overt forelimb clonus (SOTs) were engaged for a longer period in the paroxysmal masticatory activity that preceded the SOTs. This additional duration may have resulted in more damage to cells within the gelatinosus that was then evident a month later.

One biochemical candidate that may be important is the circulating levels of corticosterone. In the present studies rats with the longest SOTs exhibited the lowest concentration of corticosterone in the blood and the least amount of weight 5 days later. These results suggest that rats that displayed the fastest SOTs exhibited a more profound stress as defined by elevated levels of corticosterone and a failure to regain body weight 5 days after the seizure.

The multiple regression equation indicated that rats with the fastest SOTs also showed more damage 30 days later within the parasubiculum and the basomedial amygdala. The deleterious role of corticosteroids upon neurons exposed to trauma or to extreme metabolic challenge has been reported by Sapolsky (1994). Whether or not the enhanced neuronal dropout within the parasubiculum/basomedial amygdala but decreased necrosis within the thalamic–amygdala nuclei reflected reciprocal pathways through which the spread of the seizures might progress is not clear. This result may imply that the opening of one pathway for the paroxysmal discharges spares the structures that constitute another potential pathway. Other structures, not included in the multiple regression of analysis because it does include variables that explain the same variance, may have been involved. They would be included in the list of structures that had significant zero-order correlations with SOT.

At this time we are reticent to generalize the relationship between histomorphological patterns of damage and SOTs obtained with rats exposed to the normal procedure to those whose SOTs were experimentally manipulated by the behavioral or pharmacological procedures. If they were generalizable, then we would expect significant differences in the means in the neuronal damage within these key structures, such as the gelatinosus nucleus, as a function of the behavioral manipulations during the previous 24 h, or previous 2 weeks, or after administration of 1.0 mg/kg vs. 1.5 mg/kg of DXM. However, these manipulations might also alter the neurochemistry such that the spread of electrical seizures from the piriform cortices to other areas of the brain would follow different pathways because of the phasic or tonic changes induced by the preseizure manipulations.

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